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THE SIGNIFICANCE OF SEXUALITY[1]

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SOME years ago, as a beginner in biology, I came upon a fact which seems to be unique among animals.

The sea-squirt or tunicate, Ciona, is unable to fertilize its own eggs. A similar behavior which is very common in flowering plants is known as self-sterility. Ciona is a hermaphrodite species and discharges its eggs and sperm simultaneously into the sea water in the early morning during the breeding season. If an individual is placed in an aquarium by itself most of its eggs remain unfertilized, but if two individuals are put in the same aquarium their eggs are fertilized and promptly begin to develop.

In self-sterile plants comparable facts are well known. The pollen of a particular plant will not fertilize flowers of that plant or of a plant of exactly the same genetic constitution. But with other individuals of the same species there is complete fertility.

Since eggs and sperm produced by the same individual should be more alike in constitution than those produced by different individuals, it follows that in a successful sexual union in self-sterile animals or plants some physiological difference must exist between the uniting gametes.

Some years later I began an experimental study of the effects of inbreeding with the now famous fly, Drosophila. This was the first use ever made of Drosophila

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as a laboratory animal. Our discovery that it could be so used was followed by Morgan's much more important use of it. But with us it served the purpose of showing for the first time what the consequences were of long-continued brother-sister matings, the closest degree of inbreeding possible in a bisexual animal. We mated brother with sister for fifty-nine generations in succession. During the experiment we continued the race from generation to generation by taking pairs to breed from, from the larger broods, those which contained large progenies. Under this system no falling off was observed in the number of the progeny produced by a pair, or in the vigor of the offspring. If infertile strains were developed in the inbred population they were eliminated by our method of selecting pairs to breed from. Consequently inbreeding as such does not involve infertility or other undesirable consequences. This conclusion has since been confirmed by similar studies of the effects of inbreeding on rats and guinea-pigs, made by Dr. Helen Dean King and by Dr. Sewall Wright, respectively.

We found in the case of Drosophila that if two inbred races which were going along successfully under continuous inbreeding were crossed with each other, the progeny showed greater productiveness and vigor than either parent race. The same thing is true with Dr. King's inbred rats, with which I have been able to experiment. If you cross them with another strain of rats, the offspring, as compared with either parent race, show (1) increased rapidity of growth, (2) larger adult size, (3) earlier maturity. These are features of what is commonly known as crossbred vigor.

To what can this be ascribed? Under continuous inbreeding all individuals of the same parentage become alike. They have the same inheritance and all their gametes transmit the same genes. When an outcross is made, gametes come together which are dissimilar in genetic make-up and which produce a more vigorous reaction when they unite.

It would seem that we have here a clue to the advantage which sexual reproduction has over asexual. It can bring together gametes which are to some extent different from each other, which thus have an attraction for each other and produce a vigorous reaction when united.

Self-sterility, in Ciona and certain flowering plants, is a device which prevents the union of gametes which are just alike, and insures outcrossing with the ensuing crossbred vigor.

Let us interrupt the discussion at this point to consider what is the possible utility of sexual reproduction to organisms among which it has become almost universal. In the first place, it is not necessary or indispensable to organic life. There are many forms of life in which sexual reproduction is unknown, the bacteria and yeasts, for example. There are other living forms which apparently once reproduced sexually but have now ceased to do so, or can get along without sexuality for indefinite periods. Thus paramecium and other ciliate protozoa can be cultured for thousands of asexual generations without undergoing degeneration, if the cultural conditions are made optimum and kept uniform. Many metazoa propagate only by asexual means or by parthenogenesis.

There must then be some good and sufficient reason why sexuality ever made its appearance, for we can scarcely imagine it to have characterized life in its most primitive forms.

At the present time we find that many forms of life get along without sexual reproduction except in times of emergency. Fresh-water crustacea and rotifers multiply by producing eggs not requiring fertilization while conditions are favorable for the species, and resort to the production of fertilized eggs only when seasonal conditions become adverse, that is, the water begins to dry up or the temperature becomes too high or too low. Likewise algae may multiply vegetatively for long periods and only when conditions become unfavorable are gametes formed, the union of which in zygotes is

often followed by encystment until the environment again becomes favorable.

Since many organisms resort to sexual reproduction only in an emergency, it seems probable that sexual union may have originated as an emergency measure.

Two cells threatened with extinction unite, merge their resources. The resultant zygote has twice as great a bulk, twice as much nuclear material and twice as much plasma. Its exposed surface is, however, relatively less, so that a quiescent period or one of adverse conditions may be better met and survived. When activity is resumed a possible diversity between the cells which united may make for a more vigorous organism, the crossbred vigor principle.

The union of two individuals of the same species of one-celled organisms, however it came about in the first place, developed into a regularly repeated and orderly process which has become all but universal, so that it has required a large amount of evidence to convince biologists that sexuality was not an original property of all organic life. But we are at present pretty well agreed that sexuality is an evolved feature of life, and there is an almost endless variety of modes of sexual reproduction, some of which are so diverse that it seems almost impossible that they may have had a common origin. More probably, it is thought by most biologists, a variety of modes of sexual union originated among one-celled organisms, and from them certain ones have been handed on to the many-celled animals and plants.

A new evolutionary process, to survive and become more prevalent, must, we believe, be advantageous. We can see that sexual union would, among unicellular organisms, have the two primary advantages already mentioned, namely (1) an increase in bulk of the cell enabling it better to meet adverse conditions in a resting or encysted stage and (2) the development of crossbred vigor when the uniting cells were somewhat unlike. But disadvantages might also result. An increase in cell size with a reduction in the relative amount of surface

exposure might be an advantage during unfavorable conditions but might prove a disadvantage when conditions again became favorable and active life was resumed. For when the organism is deriving nourishment from its environment, as it must in active growth, as large a surface exposure as possible would seem to be advantageous. So we find that there is a limit of size to one-celled organisms, which are mostly microscopic creatures. When they grow large enough to become visible to the naked eye, as are paramecium and stentor among the protozoa, we find that they develop an organization within the limits of a single cell comparable with that of the metazoa, with a definite alimentary tract and a central nervous system.

But if fusion of individuals in pairs were to occur repeatedly under adverse conditions, the size of individuals would steadily increase, and would soon cease to be advantageous. In reality we find that nature has met this difficulty and holds the species size constant by uncoupling the joined individuals before a new union takes place. Cells which unite sexually are called gametes. The product of their union is called a zygote (a *joining* of cells). A gamete has ordinarily just half as many chromosomes as a zygote. The chromosomes may be taken as a convenient measure of cell size, since they can be counted. The size of the nucleus, other things being equal, depends upon how many chromosomes it contains, and the size of the nucleus in turn governs the size of the cell. The gametes of Drosophila contain each four chromosomes; the zygotes contain eight. The gametes of man contain twenty-four chromosomes; the zygotes (that is, the fertilized eggs and our bodies which develop from them) contain in each cell forty-eight chromosomes. Similar relations are found among animals and plants generally, both among the many-celled and among the one-celled organisms. Gametes we speak of as haploid creatures (having the half chromosome number); zygotes as diploid (having the double chromosome number).

In the process of forming gametes from zygotes a reduction in the number of the chromosomes takes place. The joining of two cells is unjoined. But the new individualities produced as gametes are not of necessity identical with those that united a generation before to form a zygote. Here comes in a major significance of sexuality.

Suppose that two uniting cells have each four chromosomes, A B C D and a b c d, respectively. The zygote will contain four pairs, Aa Bb Cc Dd. When reduction occurs, each new-formed gamete will receive four chromosomes, one of each pair. But we know positively from a large variety of cytological and genetic evidence that it is wholly a matter of chance *which* member of each pair goes into a particular gamete. Combinations like those of the previous generation A B C D and a b c d are possible but have only one chance in sixteen of actually occurring. Now we have very positive evidence, into which I can not go at this time, that the determiners of heredity, the genes, are contained in the chromosomes. They are very numerous and are distributed throughout the length of the chromosomes. Rarely, if ever, do two gametes in a bisexual species bear exactly the same assortment of genes, and never probably is this true of two gametes which are capable of uniting with each other to form a zygote. Hence sexual union is a constant source of genetic variability. The gametes which unite are different from each other in a certain number of genes. The zygote which they produce will thus be heterozygous (made up of dissimilar pairs of genes), and when it gives rise to a new generation of gametes the chances are that these will vary in constitution among themselves and will be different from the ancestral gametes in constitution. If only as many as four gene differences occurred between the parent gametes, the chances are only one in sixteen that a parental combination would recur. The gene differences between parents are ordinarily enormously more complex than this, and the chances are overwhelming that each zygote formed will be a unique combination of genes.

Herein then lies the major advantage of sexuality, as we see it. It furnishes an unfailing source of genetic variability. Natural selection acting upon this variable material conserves and perpetuates those gene combinations which have survival value and eliminates inferior sorts.

Along with the advantage of variability as regards genetic combinations there goes an incidental advantage difficult to estimate but of undoubted importance, the superior vigor of the heterozygous or crossbred state. A union of gametes bearing numerous dissimilar genes produces a more vigorous zygote than a union of gametes having fewer dissimilar genes. This is the important conclusion to be drawn from a large amount of experimental study of the effects of inbreeding and crossbreeding in both animals and plants. The superior vigor of crossbred animals and plants has long been known to practical breeders. In the corn belt of the Middle West it is a practice of swine breeders to cross Duroc-Jersey hogs with Berkshires for the production of more rapidly growing and so more profitable hogs for market. The Duroc-Jerseys are red coated and long bodied, prolific and of the bacon type; the Berkshire and the Poland-China breeds used in crosses with Duroc-Jerseys are black-and-white in coat color and are lard-type breeds inclined to fatness. The crossbreds are much superior to either pure breed in vigor of growth, and in the first generation are sufficiently uniform in character because heterozygous for the same assemblage of genes in which the parent breeds differ. A second generation is not produced from the crossbreds, as it would be too variable for market requirements and would fall below the first generation crossbreds in vigor, because most recombinations would involve fewer pairs of dissimilar genes.

Among farm crops, maize, the most productive of all the cereals, owes much of its superiority to hybrid vigor. If maize is closely inbred (selfed) the harvest grows less and less with each generation of inbreeding. The individual plants are feeble, grow slowly and produce very

small ears (mere nubbins) or none at all. Each inbred line becomes uniform in all genetic characters, indicating that it is growing homozygous, containing pairs of like genes. Now if two inbred lines are crossed, there is an immediate restoration of vigor, and in general the vigor is proportional to the unrelatedness of the parents, that is, to the extent that they possess unlike genes. Progressive corn-growers are beginning to act upon the advice of students of plant genetics and to plant only seed which is produced by a cross between very dissimilar types of maize, such as dent and flint varieties. By this means a maximum amount of hybrid vigor is obtained, resulting in good germination, rapid growth and early maturity, which sometimes increase the harvest of grain by as much as 10 per cent. Orchardists have long utilized unawares the hybrid vigor of chance varietal hybrids which they propagate by grafting, and forestry is beginning to experiment with similar methods. Hybrids can be produced by crossing different species of quick growing trees, such as poplars. The hybrids regularly make a more rapid growth and produce wood and lumber faster than either parent species. It is necessary to produce the hybrid only once. It can then be multiplied indefinitely by means of cuttings, each of which retains the diploid genetic constitution of the original tree with its remarkable hybrid vigor.

If dissimilarity of genes is such a stimulus to growth and source of added vigor, why not cross organisms which have *no* genes in common, and thus secure the maximum of vigor? Because such organisms will not cross. Only animals or plants of the same species will ordinarily cross with each other. We can not cross horses with cattle, or sheep with swine, or cats with dogs. Nor can we cross maize with rice, apples with peaches or figs with thistles. We come here to differences more fundamental than dissimilarity in single genes. They involve differences in the chromosomes themselves, frequently in their number, perhaps in their ground substance also. In an ordinary zygote there are two sets of

homologous chromosomes, derived, one set from the mother, the other from the father. These conjugate with each other, the I, II, III and IV chromosomes derived from the egg conjugate with the I, II, III and IV chromosomes, respectively, derived from the sperm. The conjugating chromosomes undoubtedly have many genes in common, and possibly a similarity in the ground substance of homologous chromosomes has something to do with bringing them together in synapsis.

Such similarities are apparently wanting in wide species crosses. The chromosome numbers may be different so that it is impossible for all to find synaptic mates, and even when the numbers are nearly or exactly the same, no affinity may be manifest between those of unlike parentage, indicating that they are structurally different.

Even when vigorous hybrids are formed, as in the mule, hybrid offspring of horse and ass, sterility ensues because there are not enough chromosomes which pair and disjoin at a reduction division to permit of the production of viable gametes.

Sexuality, then, can operate efficiently only when the parents have much in common. (1) They must have identical chromosome numbers so that all chromosomes can find synaptic mates at the reduction division. (2) The chromosomes of each parent must have homologues among the chromosomes of the other parent so that synapsis and disjunction may occur regularly at the reduction division. (3) This affinity for each other of homologous chromosomes must have its basis either in the nature of the ground substance of the chromosome or of its gene content or both. To what extent difference in gene content of homologous chromosomes may go without resulting in the impairment of ability to conjugate has not yet been determined but presents an interesting field for investigation.

It appears that a certain amount of diversity is advantageous and increases vigor, but too much of this good thing results in disaster. There is one condition, how-

ever, under which disaster can be avoided or even turned to good use, and a maximum of hybrid vigor secured without loss of fertility.

When a hybrid is produced between two species of plant having unlike chromosome numbers and incompatible chromosomes, such hybrids are ordinarily sterile. But in a few cases complete fertility has been observed to occur, and the hybrid plant has then perpetuated its hybrid form by seed, constituting thus a veritable new species as distinct from its parents as they from each other. A striking case is the hybrid between cabbage and radish produced by a Russian, Karpetschenko. Cytological study shows that what happens in such cases is this. The hybrid plant is not a true diploid but contains two sets of incompatible chromosomes. When cell division begins these chromosomes do not pair, but each by itself splits to furnish identical products to two daughter cells. But if now low temperature or some other disturbing cause interrupts the process before it is complete, a single cell may result which contains each chromosome in duplicate. If a plant is produced with this constitution, when the time comes for it to produce gametes, each chromosome will have as a synaptic mate its sister chromosome, and gametes will arise with the chromosome constitution of *both* parent species joined together in a permanent union. It is thus possible to produce under controlled conditions a new species of plant as a hybrid between two species which ordinarily will not cross. This process has undoubtedly been operative in the evolution of species of flowering plants in nature.

Let us now go back for a moment to the case of the self-sterile animal, Ciona, and the better known case of self-sterile plants. How can such cases have originated? To the teleologist, self-sterility looks like a device to ensure cross fertilization and thus maintain a maximum of vigor. But there is another way of looking at the matter—as a means of escape from the consequences of harboring a lethal gene. What do we mean by a lethal gene?

One which in the homozygous state causes the death of an organism. The first discovered case was that of the yellow variety of house mouse. Yellow mice never breed true but produce regularly two yellow young to one of some other color, say black. If a yellow mouse is mated to a black litter mate, half of the progeny are yellow, half are black, and the blacks breed true among themselves. The yellow parent is evidently a heterozygous dominant. Homozygous dominant yellow embryos are produced, but these die before birth, only the heterozygous yellow and the black zygotes surviving. Hence we speak of the yellow gene as a lethal. Another lethal gene is known among mice which is not fatal at quite so early a stage. This is the black-eyed-white gene. Homozygotes are born but invariably die of anemia within a few days after birth. Heterozygotes are vigorous and viable.

A self-sterile organism is one which harbors a gene lethal in the process of fertilization and which is able to perpetuate its line only by avoiding self-fertilization or fertilization by gametes bearing the same lethal gene.

Substantially this solution of the self-sterility problem seems to have been suggested first by Prell. He described successful fertilization in such cases as depending upon the existence of an "opposition factor." That is, a particular gene must always meet in fertilization a different allelomorph, never one identical with itself, or else successful fertilization was impossible. East and his pupils worked out the case experimentally. By inbreeding and selection they were able to reduce a strain of tobacco to a state in which it contained three self-sterile types (zygotes) which we may call *ab, ac* and *bc*. No further reduction could be made. Type *ab* would not fertilize itself or any other *ab* plant, but could be fertilized by either *bc* or *ac* plants. In the fertilization of a flowering plant, a pollen tube which is a haploid (gametic) structure grows down through the style which is a diploid (zygotic) structure to meet the egg cell. An *ab* plant forms pollen grains either *a* or *b* in composition. These will not grow fast enough through the *ab* tissue to

reach the egg-cell before the flower withers. Pollen *a* is slowed up by the *a* constituent of the *ab* tissue, and pollen *b* is slowed by the *b* constituent. But pollen from a *bc* or an *ac* plant will contain grains half of which are *c* in composition. These can fertilize *ab* plants producing zygotes *ac* and *bc*, respectively. So fertile unions result whenever one of the three types is pollinated with another, and a plant never produces offspring of its own type, but only of the other two types. Thus an *ab* plant produces *ac* and *bc* offspring because only *c* pollen grains can fertilize it; an *ac* plant produces *ab* and *bc* offspring because only *b* pollen can fertilize it, and a *bc* plant produces *ab* and *ac* offspring because only *a* pollen can fertilize it. In other families of self-sterile tobacco plants East found additional allelomorphs of the sterility gene, as many as fifteen, instead of the three found in the case just described.

We are now prepared to define a self-sterile plant. It is one which bears two different allelomorphs of the same gene essential to growth of the pollen tube, *both being lethal*, that is, fatal if homozygous. The original or unmutated form of such a gene is present in plants of the species which are *not* self-sterile. The lethal mutation is, like most mutations, recessive in inheritance. Thus the original state, self-fertility, is dominant over self-sterility.

Morgan has explained the self-sterility of Ciona on this same principle. The diploid tissue in this case which the haploid sperm must penetrate to effect fertilization is the envelop of follicle cells surrounding the egg. If these are removed, the block to fertilization no longer exists.

That there are not as many self-sterile animals as there are self-sterile plants is perhaps due to the fact that few animal eggs in hermaphrodite species are surrounded by follicle cell envelops which the sperm must penetrate to effect fertilization, whereas in a flowering plant fertilization can be accomplished only by growth of the haploid pollen tube through diploid tissue.

Lethal genes are very common. They are probably originating all the time. Muller and others who have been increasing the production rate of mutations by X-rays and radium in Drosophila and tobacco and barley report more lethal mutations than those of any other sort. Any harmful genetic change, if sufficiently harmful, will function as a lethal. In asexual reproduction, lethal variations are self-eliminating. The line bearing one comes to an abrupt end. In sexual reproduction a recessive lethal gene present in one gamete only of the pair which unites to form a zygote will escape notice. But if both gametes bear it, as might happen in self-fertilization or under close inbreeding, then the zygote perishes.

One of the advantages of sexuality is that it allows the race to go on and even prosper notwithstanding the presence of lethal mutations. Any number of these may be present without harmful effects so long as no one of them is allowed to become homozygous through inbreeding. Each of us no doubt harbors one or more lethal genes acquired by the race as mutations in the long centuries of exogamous organization of human societies. Let brother-sister matings be sanctioned in human society and there would be a terrible harvest of degeneracy. Not that inbreeding *produces* degeneracy, but it allows genes which are heterozygous to become homozygous, and if any of these genes are lethal or seriously harmful, a frightful result follows. Many a good family carries genes for feeble-mindedness, insanity, epilepsy and other baleful forms of inheritance which have rarely if ever become homozygous and so found expression in zygotes. But if close inbreeding occurs in any human stock, we hear the comment made that the stock is running out, inferior specimens being produced.

Corn breeders are faced with a similar situation. Maize has been cultivated for untold centuries as a cross-bred crop. If one inbreeds even the best commercial varieties, their productiveness quickly and steadily

diminishes, as one lethal or harmful gene after another becomes homozygous. The corn breeder cheerfully discards these and goes ahead with the feeble remnants of the race, hoping some day to recombine the purified but feeble strains into a vigorous one free from many of the hidden defects. But it is questionable whether this will be commercially profitable. The hidden defects do no harm so long as they remain hidden, which is insured by continuous outcrossing or breeding in large and varied populations, and even if they were eliminated by a long and expensive process of purification, there is no certainty that they or others like them might not recur again.

He would be an optimist indeed who would recommend such a process of purification of a human stock by deliberate inbreeding in hopes of some day rebuilding a stock of supermen from the feeble progeny of a few inbred villages. It is better to go on, as we have done from time immemorial, with an exogamous organization of society, for thus the maximum benefit is derived from sexuality, to which method of propagation our species is committed beyond recall.

The essential advantages of sexuality are: (1) diploid rather than haploid organization, which makes it possible for a defective or lethal gene derived from one parent to be offset by a sound gene derived from the other parent; (2) superior vigor of heterozygous as compared with homozygous genetic combinations; (3) greater genetic variability and so greater adaptability to changing environment or changing social organization.

THE PROBLEM OF THE RELATIONSHIP BETWEEN THE NUMBER AND THE SEX OF HUMAN OFFSPRING

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I. INTRODUCTION

As far as we are aware the problem of the relationship between the number of offspring produced by an individual mother and the sex of these offspring has never been adequately treated. Quite obviously the problem of the relationship between birth order and sex is closely related to the preceding.[1]

The purpose of this note is to call attention to the statistical methods which may be applied to the solution of the problem and to suggest, through the results of an analysis of available materials, the need for really adequate data for its consideration.

The most extensive series of data on the distribution of the sexes in individual families of varying size known to us is that of Geissler (1889), who has given records of the sex of 4,794,304 children nominally belonging to 998,761 families, classified according to the number of children born per family. These data suffer from two defects. First, they represent a mixture of completed and uncompleted fertilities. Second, they are weighted to an unknown extent. The records were collected over a ten-year period and it is not merely possible but practically certain that many families were represented two or more times because of the fact that an individual mother would be included each time that a birth certificate giving the sex of the child just born, together with the sex of all preceding children, was filed. Thus the number of families is less than 998,761.

[1] Both these problems have doubtless been touched upon in the literature, but it has not been practicable to make a thorough search of all the scattered publications, many of them highly speculative, on sex.

Notwithstanding these limitations, the data are probably the best available, and may serve to illustrate the methods which should be applied in the treatment of the problem.

II. RESULTS

Two methods of determining whether the proportion of the two sexes born is related to the size of the family may be employed.

(A) Instead of using the conventional sex ratio we deal with the ratio of number of male births to total births. Let $p_1 = \Sigma(m_1)/\Sigma(n_1)$, $p_2 = \Sigma(m_2)/\Sigma(n_2)$, $p_3 = \Sigma(m_3)/\Sigma(n_3)$, etc., be the ratio of the number of male births to the total number of births in families of a given size as denoted by the subscripts, and $p' = S(m)/S(n)$ be the same ratio as determined for all children of families of all sizes, where m_1, m_2, m_3, etc., represent the number of male children per family in families of 1, 2, 3, . . . children, while m is the number of males in families of any size, n is the total number of births per family, Σ denotes summation for families of a given size and S denotes summation for families of all sizes.

Two methods of procedure are now possible: (a) we may compare p_1, p_2, p_3, etc., among themselves either directly or in any modified way which may seem desirable, or (b) we may compare the values of p as determined for families of a given size with that for families of all sizes, that is, we may compare the values of p with p'.

(Aa) In the comparison of the values of p among themselves we reason as follows.

If two samples of size $\Sigma(n_j)$ and $\Sigma(n_k)$, which represent families of size j and k respectively, be drawn from an infinite population and the proportion of males, $p_j = \Sigma(m_j)/\Sigma(n_j)$, $p_k = \Sigma(m_k)/\Sigma(n_k)$, be determined for each, we must base our decision on the question as to whether they were drawn from the same or from different populations, in the precise statistical interpretation

of the term,[2] on the ratio of $p_j - p_k$ to its probable error,

$.6744898\sqrt{\sigma_{p_j}^2 + \sigma_{p_k}^2}$, where $\sigma_{p_j} = \sqrt{\Sigma(n_j)p_jq_j}/\Sigma(n_j) = \sqrt{p_jq_j/\Sigma(n_j)}$, $\sigma_{p_k} = \sqrt{\Sigma(n_k)p_kq_k}/\Sigma(n_k) = \sqrt{p_kq_k/\Sigma(n_k)}$.

The third column of Table I gives values of p for families of from one to eighteen children individually and for families of nineteen to thirty children collectively. The probable errors of these individual values may be calculated and the significance of any desired difference evaluated by comparison with its probable error.

Since mere inspection of the values of p shows approximately how the signs of the differences between the values for p for families of larger and smaller size will be distributed, it seems unnecessary to determine the 171 individual differences and their probable errors. Instead we illustrate an alternative procedure.

If p_j be the probability of male births in families of any given size, j, we may compare p_j with two other values of p, the one p^-, based on all families of a smaller size, and the other, p^+, based on all families of a larger size.

The values of p^- and p^+ are given in the fourth and fifth columns of Table I. The comparisons $p - p^-$, $p - p^+$ are made in the sixth and eighth columns. The ratios of these differences to their probable errors are given in the seventh and ninth columns.

Inspection of the differences in the sixth and eighth columns of Table I shows that, with a single exception in each series of differences, the proportion of males in families of any given size is always larger than the proportion in families of smaller size and is always smaller than the proportion in all families of larger size.

[2] All these families were drawn from the Saxon population in the ordinary usage of the term. In the more precise statistical interpretation of the term we require to determine whether the "population" of children of any given size of family differs in the frequency of male births from a "population" of any other given size of family. In other words, we have to determine whether children of families of size j differ from families of size k in the proportion of males by an amount which justified the conclusion that the difference is generally valid.

TABLE I

CRITERIA OF THE DIFFERENTIATION OF THE PROPORTION OF MALES IN FAMILIES OF VARYING SIZE.

Size of family	Number of families	p	p^-	p^+	$p-p^-$	$\frac{p-p^-}{E_{p-p^-}}$	$p-p^+$	$\frac{p-p^+}{E_{p-p^+}}$	$p-p'$	$\frac{p-p'}{E_{p-p'}}$
1	223328	.513187		.514841			−.001654	2.27	−.001581	2.26
2	223328	.513422	.513187	.514987	+.000235	0.27	−.001565	2.95	−.001346	2.79
3	179892	.514716	.513344	.515025	+.001372	2.25	−.000309	0.63	−.000052	0.12
4	148903	.513757	.513956	.515260	−.000199	0.37	−.001503	3.16	−.001011	2.46
5	120137	.514041	.513890	.515541	+.000151	0.30	−.001500	3.11	−.000727	1.78
6	95390	.513951	.513928	.515987	+.000023	0.05	−.002036	4.04	−.000817	1.94
7	72069	.514648	.513933	.516427	+.000715	1.39	−.001779	3.25	−.000120	0.27
8	53680	.514677	.514036	.517107	+.000641	1.18	−.002430	4.01	−.000091	0.18
9	38495	.515039	.514107	.518050	+.000932	1.56	−.003011	4.36	+.000271	0.49
10	26500	.517494	.514182	.518348	+.003312	4.91	−.000854	1.05	+.002726	4.27
11	16759	.516895	.514376	.519215	+.002519	3.15	−.002320	2.34	+.002127	2.75
12	10690	.516830	.514476	.520901	+.002354	2.47	−.004071	3.32	+.002062	2.21
13	6115	.519026	.514538	.522365	+.004488	3.72	−.003339	2.09	+.004258	3.58
14	3332	.520215	.514610	.524182	+.005605	3.58	−.003967	1.87	+.005447	3.50
15	1769	.521688	.514663	.526492	+.007025	3.39	−.004804	1.67	+.006920	3.35
16	913	.521495	.514700	.531691	+.006795	2.43	−.010196	2.56	+.006727	2.41
17	439	.535710	.514720	.527132	+.020990	5.39	+.008578	1.51	+.020942	5.36
18	209	.526581	.514751	.527866	+.011830	2.15	−.001285	0.15	+.011813	2.15
19–30	141	.527866	.514760		+.013106	2.06			+.013098	2.06

These differences are small, generally less than 0.01, and because of this fact their ratios to their probable errors are also in general small, notwithstanding the large number of cases included in the comparisons. When the differences are expressed as ratios to their standard deviations instead of to their probable errors their significance can be tested numerically by reference to Sheppard's tables of the probability integral (1903), reprinted by Pearson (1924). Notwithstanding the generally low values of the ratios many of the differences are sufficiently large in comparison with their probable errors that they would be considered individually significant by most statisticians. When this fact is considered in conjunction with the consistent distribution of the series of differences as a whole, there can be no possible doubt concerning the differentiation of the families of different size with respect to the proportion of male births.

(Ab) As an alternative method the deviations of the values of p for families of any given size from the value p' for all the children of the whole sample may be determined and compared with their probable errors.

The determination of the most suitable value of p' on the basis of the present data presents certain difficulties. Geissler's actual records include families of two as the minimum number. In obtaining his value for p' he took the 2,468,305 males and the 2,325,999 females of his families of from two to thirty children and added to these the 114,609 males and the 108,719 females born as the first child in families of two children.[3] Thus he based his value of p' on the 2,582,914 males out of the (weighted) total 5,017,632 births.

The procedure is, we think, not wholly justifiable. Inspection of the frequency distribution of number of children per family in Table I shows it to be wholly skew. It is, therefore, probable that had the statistics been extended to include records of mothers reporting

[3] The values of p for families of one child given in the third column of Table I were calculated from these numbers.

the sex of the first and only child the numbers would have been sensibly different from the 114,609 males and the 108,719 females as given above and as used by Geissler in the calculation of the value which we have called p'. Since there is no way in which the inadequacy of the data can be corrected after a lapse of forty years, it seems best to adopt Geissler's value of $p' = .514{,}768$ for the sex of all children. The values of p for families of given numbers of children are set forth in the third column of Table I. For families larger than eighteen the numbers are so small that it is not worth while to determine $p - p'$ for families of each individual size, but the constant has been calculated for families of from nineteen to thirty children as a combined class. Columns 10 and 11 of Table I give the results.

The differences show that while the values of $p - p'$ are small they are uniformly negative for families of from one to eight children and uniformly positive for families of from nine to thirty children. Thus males are relatively more frequent in families of large size. Examination of the actual differences shows that there is an irregular increase in the relative frequencies of males from families of one to families of from nineteen to thirty children.

In determining the statistical significance of the value of $p - p'$ as shown by the ratio of $p - p'$ to its probable error in column 11 of Table I we must note that we can not determine the value of the probable error of $p - p'$ without regard to the fact that in each case the individuals of the several subsamples represent a rather material fraction of the whole sample.

Pearson (1899) laid the foundation for the solution of the problem in his paper on properties of the hypergeometrical series, and later called attention (1906a) to the importance of correcting for the contribution made to the sample by the subsample in the evaluation of the significance of the difference between them. In another paper Pearson (1906b) dealt primarily with the problem of tests for the significance of the differences between the

mean of a subsample and of a sample. Tocher (1907, p. 335) has recognized the need for, and the possibility of the practical application of (1908, p. 143), Pearson's theory in the testing of the divergence of the relative frequency of the individuals of any special class in a given district from the relative frequency in all districts in the case of anthropometric surveys. While Pearson's methods have also been applied to the problems of individual prepotency (Harris, 1911a), of the differentiation of so-called pure-lines (Harris, 1911b) and in various other connections, their usefulness does not seem to have been generally recognized by biologists. It may not be amiss, therefore, to treat the theory briefly.

Pearson has shown that (in terms of our present data) if p' be the proportion of males, $q' = 1 - p'$ be the proportion of females, and if samples of size $\Sigma(n) = N$ be drawn from the general population of size $S(n) = M$, where N and M are commensurable, the squared standard deviation, μ_2, of numbers of males will be the second moment of the hypergeometrical series

$$\mu_2 = Npq\left(1 - \frac{N-1}{M-1}\right)$$

or when both N and M are large but commensurable,

$$\mu_2 = Npq\ (1 - N/M).$$

Here we are not concerned with the probable errors of the actual difference in number of males per sample of size N and the theoretical number as given by

$$.67449\left[Npq\left(1 - \frac{N-1}{M-1}\right)\right]^{\frac{1}{2}}$$

but with the probable error of $p - p'$,

$$E_{(p-p')} = .67449\frac{1}{N}\left[Npq\left(1 - \frac{N-1}{M-1}\right)\right]^{\frac{1}{2}} = \frac{.67449}{N}\left[Npq\left(\frac{M-N}{M-1}\right)\right]^{\frac{1}{2}}$$

which is easily evaluated.

We have, therefore, as our criterion of significance of differences in the proportion of males $(p - p')/\sigma_{(p-p')}$ or $(p - p')/E_{(p-p')}$. We must, however, recognize the fact that both the relative magnitudes of p' and $1 - p'$ and

the relative magnitudes of N and M influence the form of the integral curve to which we must appeal for the interpretation of those ratios because of the practical impossibility of working directly with the terms of the hypergeometrical series when the value of the exponent is large.

Pearson (1906a), on the basis of his own earlier work, has shown that

$$(p' - .5) \pm \left[\frac{1}{M}\left(1 + \frac{1}{M}\right)\right]^{\frac{1}{2}}$$

may be used as a criterion for selecting the appropriate curve.

Since in our present case $p' > .5 + \frac{1}{M}\left(1 + \frac{1}{M}\right)$, it would seem that Pearson's curve Type I is the proper one to use in evaluating our results. But as there is some question as to whether the normal curve may not be acceptable, the constants β_1, β_2 and κ, as determined from the moments of the hypergeometrical series (Pearson, 1899, 1906a), were calculated for the two extreme cases, families of two and of eighteen children. The results are:

	$\beta_1 = \mu_3^2/\mu_2^3$	$\beta_2 = \mu^4/\mu_2^2$	$\kappa = \frac{\beta_1(\beta_2+3)^2}{4(4\beta_2 - 3\beta_1)(2\beta_2 - 3\beta_1 - 6)}$
2	6×10^{-9}	2.999983	−.000132
18	9×10^{-7}	2.999468	−.000634

Since κ is negative the most suitable curve is Type I. The constants are, however, close approximations to those for the normal curve $\beta_1 = 0$, $\beta_2 = 3$, $\kappa = 0$. Under these circumstances we shall probably not be far wrong in relying on the areas of the probability integral in evaluating the significance of the ratios of the differences to their standard deviations.

Returning to the ratios of the several differences, $p - p'$, to their probable errors, we note that these are generally small. A number are, however, sufficiently large that they would ordinarily be regarded as individually significant. When this fact is coupled with the trend of the series of differences as a whole, there can

be no reasonable doubt that the proportion of males increases slightly with the size of the family.

These various procedures have been indicated to make the discussion of methods comprehensive. Since both of those which are illustrated lead clearly to the same biological conclusions in the present case, it is not necessary to discuss them further.

(B) As a means of dealing with all the families of all sizes simultaneously and of expressing the relationship between size of family and number of males (or females) in a single constant, the correlation between the number of children per family and the deviation of the number of male (or female) children from their probable number may be determined.

The relationship between the total number of siblings and the number of males (or females) is necessarily high, since the number of either sex represents a part of the total number.

Because of the uncertainty, indicated above, as to the exact number of families with only one child, it seems desirable to calculate the correlation for families of from one to thirty and for families of from two to thirty children separately.

The actual correlation for families of from one to thirty children is

$$r_{nm} = .8188 \pm .0003$$

while for families of from two to thirty children it is

$$r_{nm} = .7805 \pm .0003.$$

If the number of males constitutes a definite proportion $p = S(m)/[S(m) + S(f)] = S(m)/S(n)$ of all births and if this proportion were constant for all values of n, the correlation $r_{n(m\,-\,pn)}$ should be 0. If, however, male births occur relatively more frequently in larger sibships than in all sibships the correlations should be positive.

The formula required is that measuring the correlation between a variable and the deviation of a dependent variable from its probable value (Harris, 1909, 1918b). This method has already been applied in a number of

biological investigations which need not be cited here except to note that in 1918 it was employed in a study which led to the demonstration of no close relationship between number per litter and sex in swine (Harris, 1918b) and in two other papers which may have a general bearing on the problem of the physiology of sex since they have to do with the relationship between the total number of sporophylls and the number of macro- and micro-sporophylls in *Arisarum* (Harris, 1916) and *Ficaria* (Harris, 1918a) and between the total number of florets and the number of pistillate and hermaphrodite florets in *Homogyne* (Harris, 1918b).

In our present notation

$$r_{nz} = \frac{r_{nm} - V_n/V_m}{\sqrt{1 - r_{nm}^2 + (r_{nm} - V_n/V_m)^2}}$$

where z is to be read as the deviation of the number of males from its probable value and V_n, V_m are the coefficients of variation of number of children and number of male children per family. The coefficient for families of one to thirty children is

$$r_{nz} = +.0062 \pm .0006$$

while for families of two to thirty children it is

$$r_{nz} = +.0068 \pm .0007.$$

The constants indicate that in the larger families males are slightly more numerous than would be expected. The probable error has been calculated on the assumption that it is legitimate to use the weighted number of families as N, or specifically on the bases of $N = 1{,}222{,}089$ for families of one to thirty and $N = 998{,}761$ for families of two to thirty children. The ratio of the coefficient to its probable error is 10.33 for families of one to thirty children and 9.71 for families of two to thirty children. The ratios leave no question concerning the statistical significance of the relationships.

The regression of the deviation of the number of males from its probable value on the number of children per family for families of one to thirty is

$$z = -.0092 + .0022\,n$$

while for families of two to thirty children it is

$$z = -.0135 + .0028\,n.$$

These equations show how slight the actual relationship between size of the sibship and the sex of the siblings is.

III. Summary and Conclusions

The primary purpose of this article has been to indicate the statistical procedures which may be applied in the investigation of the problem of the relationship between the number and the sex of human offspring.

It is here shown, by the application of these methods to the analysis of records of the distribution of the sexes in nearly a million German families with a total of about five million children, that the proportion of male births increases *slightly* with number of births per family.

The relationship between the size of the sibship and the sex of the offspring is of a very low order of magnitude, but is unquestionably significant for the series of data in hand. There is, of course, a possibility of some undetermined source of bias in the data, and for this reason similar constants should be determined from other and wholly independent series of records.

The demonstration of the relationship is of importance in showing that there exists a real biological problem, or more properly a group of problems, concerning the relationship between the fertility of the parents and the sex of the offspring.

In this investigation attention has been limited to the relationship between the size of the sibship and the sex of the siblings because the data for a consideration of the possible relationship between the birth order and the sex of the child are inadequate. It is quite possible that the relationship here demonstrated between the members in the sibship and the sex of the siblings is merely the resultant of a correlation between birth order and sex and

birth order and size of sibship. We have, as far as we are aware, no exact knowledge of the correlation between sex and birth order in human families. That measurable characteristics of the new-born infant may be related to the age of the parent and to pregnancy order and birth order has already been shown (Harris, 1926a, 1926b).

The present findings should not lead prematurely to the conclusion that either size of sibship or birth order as such has a direct influence on sex. At least two alternatives must be considered before such a conclusion is drawn.

First, both birth order and number of offspring may be functions of more fundamental variables with which sex may also be correlated. That number of offspring is correlated with other physiological variables is indicated by the work of Beeton, Yule and Pearson (1900) and of Powys (1901, 1905).

Egg production in the fowl is in certain obvious regards analogous to (though not homologous with) childbirth in man. In both cases there is a relatively large material drain on the parent organism. Recent studies have not merely indicated that there is a differential death-rate in the case of standard breeds of the fowl (Harris, 1928b), but show that there is a relationship between antecedent egg production and mortality (Harris, 1926c, 1927, 1928a). While the relationship is doubtless in part due to pathological factors, there are reasons to believe that it is to some extent dependent upon the constitution of the individual organisms.

Second, it must not be forgotten that differential mortality may influence the ratio of the number of males (or females) to the total number of offspring. The sex ratio of new-born infants does not necessarily represent the sex ratio of the zygotes at an earlier stage of development. For a final solution of the problem treated in this paper, a consideration of intrauterine mortality is essential.

The methods here suggested are of necessity applied to a series of data which is not adequate for a full treatment of the problem. As far as we are aware wholly suitable materials are not available on the large scale which is desirable. It is our hope that the results of this preliminary investigation may result in the uncovering of some additional published series of data or in their accumulation by some worker in institutions where they can be obtained.

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FACTORIAL BALANCE IN THE DETERMINATION OF FRUIT SHAPE IN CUCURBITA

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IN so far as organic form has a specific genetic basis it must evidently be subject to the laws which control heredity in general; and in the last analysis it is therefore presumably determined, according to current theory, by the activity of genes. That shapes are inherited has repeatedly been proved, but as to the relationship which exists between a given shape and its basis in the genetic constitution of the organism, very little is known. This is one of the major problems in morphogenesis and is obviously of much significance to morphology in general.

The first step toward its solution in any given case is to make a detailed Mendelian analysis of the form in question, based on quantitative rather than merely descriptive data. Not many such analyses have thus far been attempted. Among these are the studies on the inheritance of fruit shape in *Cucurbita pepo* made by the senior author of the present paper. In this species it was found (1922) that the flattened or "disk"-like type of fruit apparently differs from the spherical or isodiametric type by a single genetic factor and that a clear-cut segregation occurs in the F_2 of crosses between disk and sphere. Later (1927) it was shown that the typical disk shape is due to the cumulative effect of two dominant "flattening" factors, each of which, if present alone, produces an essentially spherical fruit. Two such spheres, possessing different flattening factors, give when crossed an F_1 entirely of disk-fruited plants; and the F_2 shows approximately 9/16 disk, 6/16 sphere and 1/16 elongate fruits, the last being the double recessives.

In previous papers the presence of various additional factors for fruit shape in this species was suggested. The operation of one class of these is reported in the present paper. It should again be emphasized that the

factors here discussed seem to be those which control dimensional ratios, and that they have no relation to the actual magnitudes of the dimensions themselves.

METHODS AND MATERIALS

The same methods of analysis have here been employed as in previous work. The parent lines were inbred for from three to ten generations and (with exceptions to be noted) were apparently essentially homozygous. The length of the fruit was measured along its axis (from stalk to corolla scar) and the width at its widest point at right angles to this axis. Its shape index was determined by dividing the longer dimension by the shorter, this quotient being followed by the letter W or L to indicate whether the fruit was wider than long or longer than wide (the index of 1.0 representing an isodiametric fruit). The populations were classified according to the logarithms of these shape indices, for reasons previously discussed.

The lines used in the preceding studies, together with others as yet undescribed, were employed in the present investigation. Line 103, an inbred strain with isodiametric fruits and grown since 1916, has been shown to possess factor *A*, one of the two "flatteners" previously identified, and thus has the genotype *AAbb*. Line 22, also long established, is somewhat more elongate, about 1.5 L, and possesses a second and independent flattener, *B*, thus having the genotype *aaBB*. The F_1 between these is a flat disk with an index of about 3.0 W; and the F_2 (as indicated above) consists of about 9/16 *AB* (disk), 3/16 *Ab* (sphere), 3/16 *aB* (sphere) and 1/16 *ab* (elongate). These *ab* plants have markedly elongate fruits, with an index of about 3.0 L. When crossed with *A* or *B* spheres they are recessive and show monohybrid segregation in F_2. Evidence from back crosses and from other sources all confirms the hypothesis that these elongates have the recessive allelomorphs of the *A* and *B* flattening factors.

Other elongate-fruited plants, however, with shape indices much like the *ab* individuals, but unrelated to

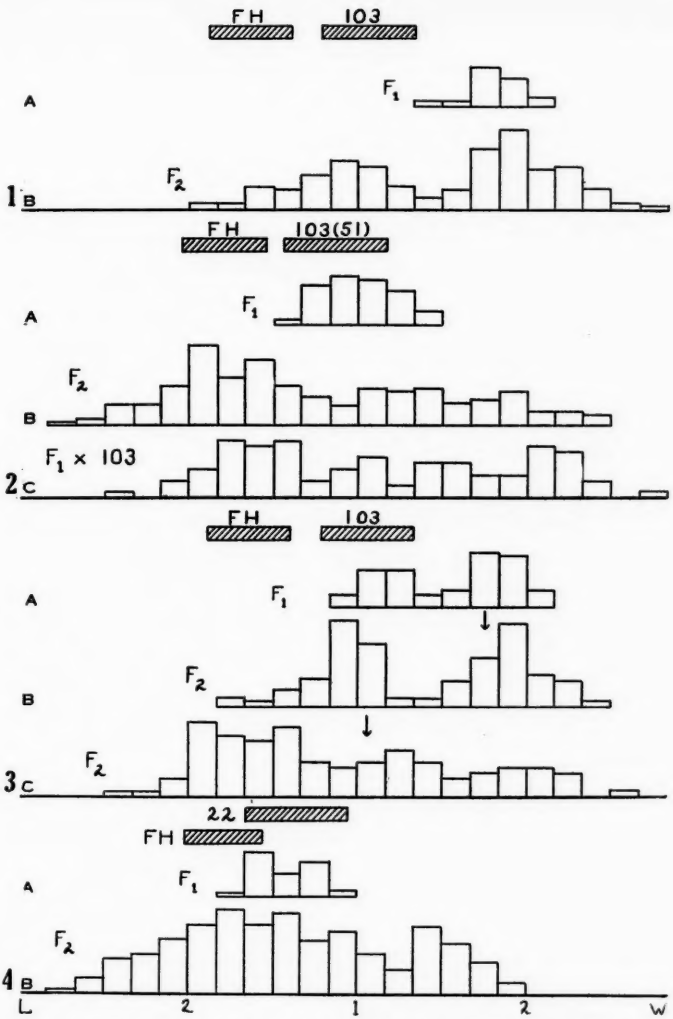

FIG. 1. Shape indices of parent lines, F_1 (*A*), and F_2 (*B*) of first cross between FH and 103.

FIG. 2. Shape indices of parent lines, F_1 (*A*), and F_2 (*B*) of most of the later crosses between FH and 103 or similar lines, *C*, back cross of $F_1 \times 103$.

FIG. 3. Shape indices of parent lines and F_1 (*A*) of cross between FH and 103 in which the F_1 is bimodal. *B*, F_2 from the flatter mode of the F_1 (index of F_1 parent shown by arrow). *C*, F_2 from the more elongate mode of the F_1 (index of F_1 parent shown by arrow).

FIG. 4. Shape indices of parent lines, F_1 (*A*) and F_2 (*B*) of cross between FH and 22. The values of the shape indices are shown below the last figure. Elongation is plotted to the left and flattening to the right.

them and derived from various commercial strains, behave in an entirely different fashion when crossed with spheres and disks, the F_1 usually being intermediate in shape and the F_2 showing much less evidence of segregation. It is therefore clear that elongate-fruited plants essentially similar phenotypically may be radically different in their genetic constitution, and an attempt is made in the present paper to arrive at a satisfactory analysis of some of these differences.

RESULTS

The problem is evidently a complex one, for no two of the elongate lines which have been tested with standard stocks behave in the same manner. Certain of them are simpler than others, however, and a study of these has provided the clue by which it is believed that an analysis of all may ultimately be made.

One of these elongate types is line FH, which was originally derived from the "Fordhook" variety of summer squash developed by Burpee. It has an elongate, white, slightly ribbed fruit with a shape index of about 2.0 L. Our strain was obtained from commercial seed in 1920 and has been inbred since that time. In 1921 a plant from line FH, which at that time was slightly less elongate than it has since become, was crossed with line 103 (index about 1.0) and produced an F_1 in which the fruit was very much flatter than in either parent, essentially a thick disk with an index of nearly 2.0 W (Fig. 1 *A*). In an F_2 of 332 individuals (Fig. 1 *B*) this gave a distinctly bimodal population, about 9/16 of which had disk-shaped fruits and 7/16 spherical or long-spherical ones. This suggested the operation of two complementary or cumulative flattening factors, an interpretation which was worked out in detail later for another cross. All subsequent attempts to repeat this result with crosses between line FH and line 103 or the essentially similar lines 105 and 51 were unsuccessful. They typically showed (Fig. 2 *A* and *B*) an F_1 much more elongate than in the previous case and of about the index of the iso-

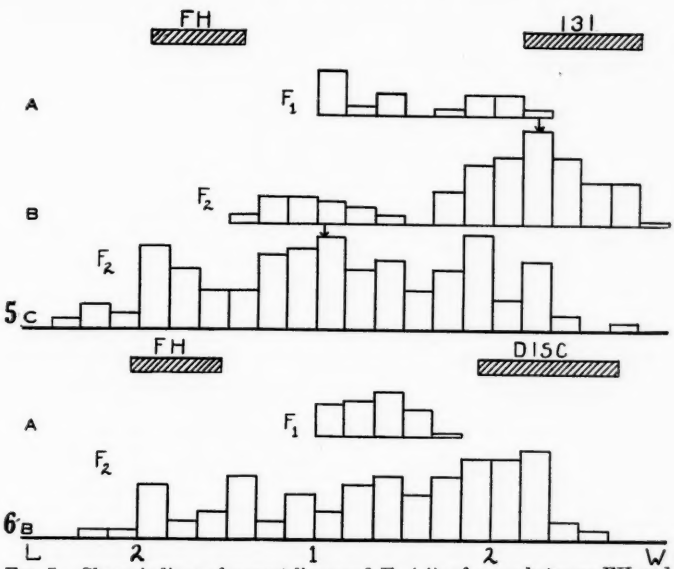

FIG. 5. Shape indices of parent lines and F_1 (*A*) of cross between FH and 131. *B* and *C*, F_2 generations from the flatter and from the more elongate modes of the F_1, respectively.

FIG. 6. Shape indices of parent lines, F_1 (*A*), and F_2 (*B*) of cross between FH and several similar and representative disk-fruited lines. Data from three crosses thrown together.

diametric parent; and an F_2 with a large number of Fordhook-like elongates, a considerable proportion of spheres and a relatively small group of disks of the same index and breeding behavior as the disks produced in the first cross. This result has been obtained with essential uniformity in eight crosses made in four seasons and involving about 450 individuals. Since line 103 is known to have become essentially homozygous very early, it is evident that the FH parent plant in the first cross described was genotypically different from those used later.

A suggestion as to the cause of this difference was provided by the result of one cross between lines FH and 103 made in 1922. The F_1 here was distinctly bimodal (Fig. 3 *A*), half of it being essentially like the F_1 in the first cross mentioned above (disk) and the rest like the

F_1 of the later crosses (isodiametric). The F_2 generations grown from each of these two modes are shown in Fig. 3 *B* and *C*. It is clear that the F_2 from the disk-like F_1 (*B*) is definitely bimodal and that its modes and its relative proportions correspond very closely indeed to those of the first cross, shown in Fig. 1 *B*. The F_2 from the isodiametric portion of the F_1 (*A*), however, corresponds very closely to those from the later crosses of FH $\times$ 103 (or 51), shown in Fig. 2*B*.

The explanation at once suggests itself that in this particular cross the FH parent was heterozygous for a single factor opposite in effect to the flattening factors already shown to be present in both lines FH and 103. That portion of the F_1 which lacked this inhibiting or elongating factor was essentially like the F_1 in the first cross, and that portion possessing it (of course in heterozygous condition) was like the F_1 in the later crosses. The FH plant first used in these crosses evidently lacked this factor entirely.

This conclusion is strengthened by the results of several other crosses involving line FH in which the F_1 was clearly bimodal. One of these was made in 1922 between line FH and line 131, the latter a pure strain of disk-fruited plants with an index of about 3.0 W. The results of this cross are shown in Fig. 5. The F_1 here is also clearly bimodal, half being disk-shaped and the other half approximately isodiametric. The F_2 grown from the disk-like F_1 (Fig. 5 *B*) showed a simple 3:1 segregation into disks and spheres; that from the isodiametric F_1 (Fig. 5 *C*) showed a complex segregation somewhat like those in the F_2 of FH $\times$ 103 and similar lines (Figs. 2 *B* and 3 *C*) but with a considerably higher proportion of disk-fruited plants.

The behavior of line FH (in its later and homozygous condition) in crosses with line 22, which possesses the flattening factor *B*, is significant and is shown in Fig. 4. Here the F_1 is essentially like line 22 but in the F_2, just as in crosses between FH and 103, there are a considerable proportion of disk-fruited plants. These are not as

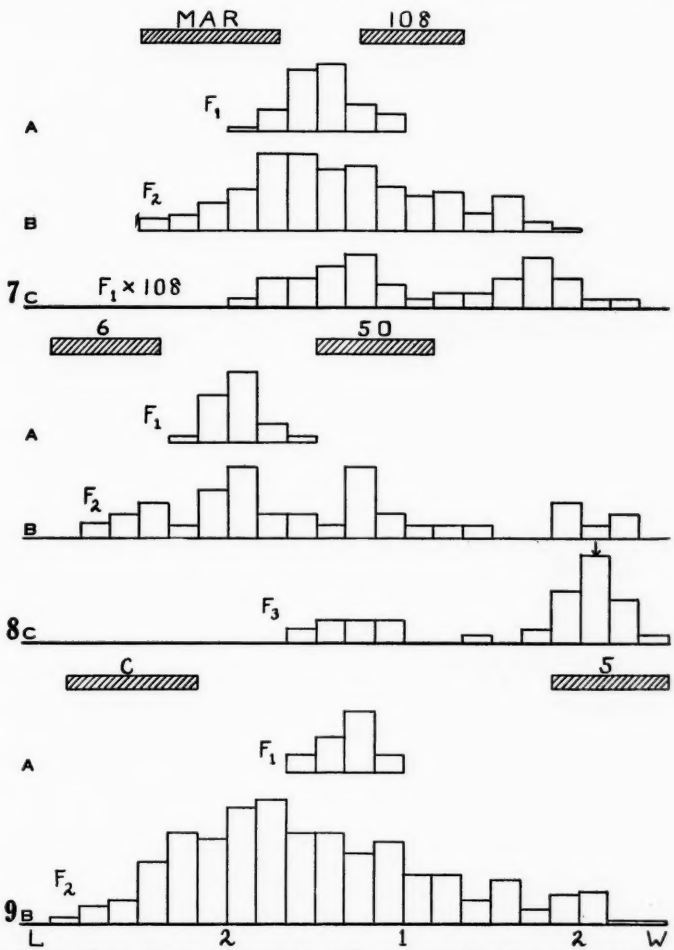

FIG. 7. Shape indices of parent lines, F_1 (*A*), and F_2 (*B*) of cross between MAR and 108 (similar to 103). *C*, back cross of $F_1 \times 108$.

FIG. 8. Shape indices of parent lines, F_1 (*A*), and F_2 (*B*) of cross between 6 and 50 (similar to 103). *C*, F_3 from disk-fruited F_2 plant the index of which is shown by the arrow.

FIG. 9. Shape indices of parent lines, F_1 (*A*), and F_2 (*B*) of cross between C and 5.

flat as the disks from the cross just mentioned, presumably because the flattening factor *B*, brought in by line 22, is not as powerful as *A*, brought in by line 103; a sug-

gestion which is supported by the fact that line 22 itself does not show as much flattening as does line 103.

These various results may be explained by assuming the operation of four major factors, three for flattening and the fourth opposite in its effect. Evidently, line FH must contain a flattening factor which, combined with factor *A*, from line 103, produces a cumulative flattening effect (as in the case previously reported for factors *A* and *B*), and disk-fruited plants result. These may appear either directly in the F_1 (Fig. 1) or as segregating group in the F_2 (Fig. 2). Furthermore, this flattener introduced by line FH can not be identical with factor *B*, for when FH is crossed with line 22 (which possesses *B*) a segregating group of disk-fruited plants also appears in the F_2 (Fig. 4). This indicates that factor *B* and the flattening factor present in line FH are cumulative in their effect, a result which would be impossible if they were identical. This third flattening factor, introduced by line FH, we shall call *C*. The genotype of line 103 (and related lines) for these flatteners is thus *AAbbcc;* of line 22, *aaBBcc,* and of line FH, *aabbCC.*

The results of the cross between line FH and line 131 (disk-fruited) shown in Fig. 5 seem to prove that line 131 differs in only a single factor from line FH, since the F_2 derived from the disk-shaped portion of the F_1 shows a monohybrid segregation. This line is therefore probably *aaBBCC* genotypically, rather than *AABBcc,* as most other disk-fruited lines have been shown to be. Later crosses of line 131 with both *Ab* and *aB* spheres indicate that this hypothesis is correct.

Of perhaps more significance is the fact that in addition to this third flattening factor, line FH contains a factor opposite in effect to these various flatteners. Whether this is merely an inhibitor of flattening or a real "elongating" factor will be discussed later. At least, it prevents the cumulative effect of two flattening factors (to produce a disk) when these are brought together in crosses between line FH and both *Ab* and *aB* spheres. We shall designate this factor as *I*. Line FH would thus

have the genotype *aabbCCII*. The F_1 of a cross between this and line 103 (*AAbbccii*) would thus be *AabbCcIi;* and although there are two flattening factors here, the presence of *I*, even in heterozygous condition, results in a shape little or no flatter than a sphere. In the F_2, however, 1/4 of the plants should lack this factor, and of this fraction, 9/16 should possess both *A* and *C* and thus have disk-shaped fruits. These disks (the *ACi* plants) should therefore constitute 9/64 of the F_2, an expectation which is seen to be approximately realized in the F_2 generations shown in Figs. 2 *B* and 3 *C*, although the segregation is not sharp. The F_2 populations figured comprise 315 individuals. If one counts as disk-fruited plants all those of index 1.59 W or wider (beginning four classes to the right of 1.0) there are 54 such plants. On the basis of an expectation of 9/64 there should be 44. The excess is probably to be accounted for through the operation of minor flattening factors (known to be present in several lines) in addition to the two major ones. A similar result is evident in the F_2 of FH $\times$ 22 (Fig. 4).

The larger proportion of disks in the F_2 from a cross of line FH with line 131 (Fig. 5 *C*) is to be expected, for the F_1 would presumably be *aaBbCCIi*, since *C* is common to both FH and 131; and there should thus be 3/16 disk plants (*ACi*) in the F_2 instead of 9/64.

The segregation of the other fruit types in the F_2 generations of crosses between line FH and lines 103 and 22 (Figs. 2 *B*, 3 *C* and 4 *B*) is not much sharper than that of the disks. In both F_2 generations from FH $\times$ 103 there is a rather large group which has approximately the index of line FH (elongate) and a smaller one essentially like the F_1 (isodiametric). This may be explained by assuming that factor *I* in its homozygous state can counteract the effect of both flatteners and produce an elongate fruit, and that in its heterozygous condition it can counteract a single flattener to produce a very similar result. Of the F_2, 1/4 should be *II;* and of the 1/2 which are heterozygous for *I*, 7/16 should possess only one flattener; and there should be 1/64 of *abi* elongate

plants. This gives, on the above assumption, nearly 1/2 of the F_2 which should be more elongate than the F_1, a result in essential agreement with the actual F_2 generations shown in the figures. Plants resembling phenotypically the F_1 or the spherical parent, and thus with essentially isodiametric fruits (either heterozygous for *I* and possessing both *A* and *C*, or lacking *I* and possessing either *A* or *C* alone), should comprise 9/16 of 1/2 plus 6/16 of 1/4, or 3/8 altogether, a result which is also essentially realized. Thus theoretically we should expect about 1/2 elongate plants, 3/8 isodiametric ones, and 9/64 disks, approximately the results obtained in crosses between lines FH and 103 and 22 as shown in Figs. 2 *B*, 3 *C* and 4 *B*.

This hypothesis was also tested by a series of back crosses the result of one of which is shown in Fig. 2 *C*. Here the F_1 of the cross of FH $\times$ 103 (*AaCcIi*) was crossed back on an *A* sphere (*AAccii*). We should evidently expect four classes of plants as to fruit shape (*I* being heterozygous in every case): *ACI* plants, like the F_1; *AcI*, more elongate than the F_1 since only one flattener is present; *ACi*, disk-fruited plants, and *Aci*, isodiametric plants, like line 103. The results, as in all these crosses, are doubtless complicated by the operation of minor flatteners which are known to be present, but they agree reasonably well with the theoretical expectations outlined above.

In the cross between line FH and disk line 131 (*aaBBCCII*) there are markedly fewer elongates and more isodiametrics and disks (in the F_2 from the F_1 containing *I*, Fig. 5 *C*) than in crosses of FH with 103, as is to be expected from the fact that one of the flatteners (*C*) is present in both parents and that there is a proportionally larger representation of flattening factors in the F_2. In crosses between line FH (*aabbCCII*) and most other disks (*AABBccii*) there is still more pronounced shifting of the shape indices toward the relatively more flattened types. The F_1 (*AaBbCcIi*) (Fig. 6 *A*) is considerably flatter than in previous crosses, and

in the F_2 (Fig. 6 *B*) there is a much smaller group of plants resembling the FH parent and a much larger proportion of disks. These facts are evidently to be explained by the presence of three independent flatteners, *A, B* and *C,* instead of two, with the result that the balance between flattening and elongation has been shifted considerably. The results shown in the figures are in essential agreement with these theoretical expectations.

To summarize briefly, therefore, we may conclude that line FH, with fruit rather elongate in shape, possesses a flattening factor different from, and inherited independently of, the two flattening factors previously shown to be present in lines 103 and 22, respectively, and possesses also a factor markedly opposite to these in its effect and inherited independently of them.

This hypothesis of a series of "flattening" and "elongating" factors, independent in inheritance, also explains satisfactorily the results of crosses involving other lines with elongate fruits and has been tested by an extensive series of F_2, F_3, and back-cross progenies, a few of which will now be described.

Line MAR $\times$ *Line 108.*—The results of this cross are shown in Fig. 7 *A* and *B*. Line MAR is derived from a race of "vegetable marrow" squash, having oblong fruits with an index of a little over 2.0 L. Line 108 is derived from line 103 and has a similar genotype (*AAbbccii*). The F_1 is approximately intermediate in shape between the parent types. In F_2 a small group of rather thick but undoubted disks appears, just as in the preceding crosses, indicating that line MAR carries a flattening factor (or factors) different from *A*. Whether this is identical with factors *B* or *C* has not been definitely determined by breeding tests, but the relatively slight flattening of the disk-shaped fruits suggests that it may be different from any of these and markedly weaker in its effect, and we may provisionally name it *D*.

The intermediate character of the F_1 indicates that line MAR carries an inhibiting or elongating factor, and the proportion of disk-fruited plants in the F_2 suggests that

only one such factor (or at least only one conspicuous one) is present. This conclusion is confirmed by the appearance of the back cross of the F_1 on line 108 (Fig 7 *C*) which is much more sharply bimodal than the corresponding progeny from FH $\times$ 103 (Fig. 2 *C*) and is also more compact. One group covers approximately the range of the F_1 and presumably contains the *I* factor. It is apparently made up of the *ADI* and *AdI* individuals. The other group, presumably lacking *I*, are spheres and flat disks and are apparently *ADi* and *Adi*. The sharply bimodal character of this back-cross may be explained by assuming that the elongating factor brought in by line MAR has a relatively greater effect in proportion to that of the flattening factor than was the case in the previous back cross described.

Line 6 $\times$ *Line 50.*—Line 6 is a markedly elongate type which was segregated six years ago from a cross between a "crookneck" and line 103. It has a mean index of about 3.0 L. Line 50 is genetically like 103 in possessing the *A* factor without *B* or *C*, but is somewhat more elongate, a circumstance probably due to the absence of certain minor flatteners known to be present in 103. The F_1 and F_2 of a cross between these two lines are shown in Fig. 8 *A* and *B*. The F_1 is intermediate in shape. In the F_2 there is a small but sharply segregated group of disk-fruited plants, thus indicating that line 6, like lines FH and MAR, has in its genotype a flattening factor different from *A* (introduced by line 50) and cumulative with this in its effect. A cross between line 6 and line 22 (the latter possessing factor *B*) showed no disk-fruited plants in F_2, thus showing that the flattener present in line 6 is probably identical with *B*. The intermediate character of the F_1 and the occurrence of disks in the F_2 also indicate the presence of an inhibiting or elongating factor in line 6, and the proportion of the F_2 disks shows that there is probably only one of these.

The F_2 as a whole shows several modes, one resembling line 6, one the F_1, one line 50, one less well marked and somewhat flatter than this and the last the disk-fruited

plants just discussed. These disks are evidently the 9/64 *ABi* plants to be expected in the F_2 between *aBI* and *Abi*. An F_3 progeny grown from one of these (Fig. 8 *C*) shows a sharp 3:1 segregation, the parent disk here evidently being homozygous for one of the flatteners and heterozygous for the other. In the non-disk portion of the F_2 segregation is evident, but just what is the genetic constitution of each of the groups it is difficult to determine. As compared with the F_2 of the cross between line FH and line 103 (Figs. 2*B* and 3 *C*) there are relatively fewer individuals (much less than 1/4) in which the shape of the more elongate parent is recovered, and relatively more which resemble the F_1. Evidently the plants homozygous for *I* are by no means all as elongate as line 6. It thus seems probable that this elongating factor is not identical with the one present in line FH, but that it is both more effective, since it produces a more elongate fruit shape in line 6 and the F_2 derived therefrom; and less dominant, since there is evidently a greater difference between its effect in homozygous and in heterozygous condition. Critical breeding evidence as to the relationship between the elongating factors in lines FH, MAR and 6 is not yet available. Using the same symbol for the elongating factor as before, however, we may suggest that the F_2 groups resembling line 6 are *AbII* and *aBII,* homozygous for *I* but possessing only one flattener; those resembling the F_1, *ABII* and *ABIi,* possessing two flatteners; those resembling line 50 and the adjoining wider group, *AbIi, aBIi, Abii* and *aBii;* and of course the disks *ABii.* The theoretical expectation on this basis is met fairly well by the actual distribution.

We may therefore conclude, at least provisionally, that the shape genotypes for the lines discussed are as follows: FH, *aabbCCddII;* MAR, *aabbccDDII;* 6, *aaBBccddII;* 103, *AAbbccddii,* and 22, *aaBBccddii.*

In all these cases the results can be explained satisfactorily on the assumption that a single major inhibiting or elongating factor is operative. In many other elongate lines, however, the results can not be interpreted in

such a simple fashion. Line C, for example, which has been derived from a commercial "crookneck" type, has approximately the same shape and dimensions as line 6, but when it is crossed with standard spheres and disks, the results are very different indeed. Fig. 9 *A* and *B* shows the F_1 and F_2 of a cross between line C and a typical disk, line 5. The F_1 is intermediate between the two parent types. In the F_2 there are very few individuals in which the disk shape of line 5 is recovered, and the distribution of the whole population is a relatively smooth one with little indication of segregation. Both these facts can be explained by assuming that line C contains two (or probably more) inhibiting or elongating factors instead of one, as do the lines previously discussed. Whenever line C is crossed with other disks or isodiametric types the same results appear and very few non-elongate parental forms are recovered in F_2. A number of other elongate lines behave in the same way as line C and thus evidently possess several inhibiting or elongating factors.

Just how these factors operate can not well be determined at present. The results may be explained in fairly adequate fashion by assuming that they do not have a positive effect themselves but merely inhibit the operation of the factors which tend to flatten fruit shape. This is further suggested by the fact that in no case do these factors seem to be cumulative in their effect (as are flattening factors) for the F_1 of a cross seems never to be more elongate than either parent. On the other hand, it is quite possible that in some or all cases there may be a positive elongating effect. It is rather difficult to demonstrate this since the recessive allelomorphs of the flattening factors thus far identified tend to elongate fruit shape, and thus the independent effect of the *I* factors in the absence of flatteners would be difficult to see. It is highly probable that these elongating factors differ in their strength, as do the flatteners, and that in addition to one or more major factors of this type there may be a series of minor ones.

The important point to emphasize, however, is that these factors are opposite in effect to the flatteners and that the fruit shape produced by any given genotype is determined by the balance between factors tending to make it flat and others working in the opposite direction. Shapes which are phenotypically similar may be genotypically very different. Thus a very elongate shape may be due either to the absence of dominant flattening factors (*aabb* elongates) or to the operation of one or more inhibiting or elongating factors (lines 6, C and others). In the same way, a spherical fruit may result from a single flattener, as in lines 103 and 22; or it may be due to two or more flatteners combined with elongating factors, as is the case in a spherical line which has recently been developed from a cross between line C and a disk, and which breeds entirely differently from *Ab* or *aB* spheres. This concept of balance is in line with the results of other investigators who find, in an increasing number of plant and animal species, that a single trait is often the resultant of the combined action of a group of factors differing in the direction of their effects.

The results set forth in the present paper give further evidence for the existence of genetic factors which determine shape. It should again be emphasized that these are not dimensional factors governing the rate of growth in certain directions but that they operate quite independently of actual size and seem rather to control the correlations which exist between growth in one direction or dimension and growth in another. This distinction between size and shape factors is fundamental.

The concept that growth correlation is not a relatively simple physiological phenomenon but is controlled by a series of particulate entities of some sort, various in the degree and direction of their influence, and that any given shape which appears during the course of development results from a genotypic balance between these factors, serves as a useful point of approach to the problem of the genesis of organic form.

SUMMARY

1. Several independent genetic factors tending to flatten fruit shape in *Cucurbita* have been isolated. Their recessive allelomorphs produce various elongate shapes.

2. Other elongate types are evidently not due to factors of this sort but to others which either produce shape elongation directly or inhibit the operation of flattening factors.

3. Genetic analyses of certain pure lines of *Cucurbita* with elongate fruits, based on a study of crosses between these and flatter lines of known constitution, are described. These indicate that in certain lines there is one elongating factor of this sort, whereas others evidently possess two or more.

4. A specific fruit shape seems to be the resultant of a balance between a series of factors differing in the degree and direction of their effect. Shapes which are phenotypically identical may be very diverse genotypically.

5. These shape factors evidently operate through their influence on growth correlations.

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SOME PROBLEMS IN THE UTILIZATION OF INBRED STRAINS OF CORN (*ZEA MAYS*)[1]

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ENGROSSED as we are with the details of the undertaking, we may fail to realize that the program of corn improvement now under way in America is one of the most significant ventures in the history of plant breeding. It is unique no less for its magnitude than for the novelty of the methods employed. The movement is founded upon the illuminating Mendelian analysis which has been made of the composition of populations of naturally crossbred organisms. Beginning with the work of Shull and of East, when the field of genetics was still comparatively virgin soil, and continuing with Jones and others, entirely new objectives in corn breeding have been revealed and the means whereby they may be attained put in our hands. Through the agency of fundamental research in heredity we have emerged from a stalemate in the development of our most important economic plant. Now an advance has set in with corn and other crops of its class which can be compared only with the activity which followed the discovery of constant lines by Nilsson and Johannsen's establishment of the genotype doctrine.

The modern corn breeder is faced with a difficult task. Through the application of a drastic form of inbreeding accompanied by selection he has undertaken to rid the plant of the undesirable germ-plasm which it carries. In attempting to purge a variety of its germinal dross by this means, however, the stock is severed into a number of distinct lines each of comparatively low yield and, there-

[1] Paper read as a part of the symposium on "Recent Developments in Research with Corn" at the joint session of Section O of the American Association for the Advancement of Science, the American Society of Agronomy and the Geneticists Interested in Agriculture, at Des Moines, Iowa, December 28, 1929. Papers from the department of genetics, Agricultural Experiment Station, University of Wisconsin, No. 107. Published with the approval of the director of the station.

fore, of little immediate worth. But these subgroups may embody among them the most valuable elements in the original variety. The problem with which the breeder is confronted is how to compose from these potentially valuable inbred strains a single stock of high commercial worth.

IMPORTANCE OF ELIMINATING UNDESIRABLE GENES

I should like to digress somewhat from the main topic for a moment to mention a point concerning the ultimate development of the present system of corn breeding which seems worthy of notice. At present it is considered that acceptable increases in yield through the use of inbred strains are obtained only in certain unstable combinations which must be continually made anew. In other words, the product which is to be furnished the grower is not a self-perpetuating stock. The very significant gains which are to be made by this procedure are well known, and corn breeders are on sound ground in applying themselves now to the task of putting it on a practical basis.

We should not overlook the fact, however, that in resorting to crossing to secure vigor we are, in a sense, temporizing with a condition in the stock which it was the purpose of inbreeding and selection to remove. Hybridization is employed to atone for a partial success in ridding the parent lines of their defective germ-plasm. The point I should like to emphasize is that the supreme merit of the method of corn improvement now being practiced lies in the opportunity it affords for eliminating undesirable genes. I fear that sometimes we are inclined to think that our task is finished when a way is found of covering them up.

The ultimate objective in corn breeding lies beyond the stage when high yield is to be obtained only in a hybrid between two stocks. In saying this it is not intended to imply that the method of using crossed corn is going to prove of mere passing interest. It appears destined to become firmly intrenched as an important method of seed

production for a long period of time. Eventually, however, it should be possible to combine in a relatively true-breeding line the favorable factors for development now scattered in several. Then the grower may be furnished with a high yielding stock which is self-perpetuating. The realization of such an objective, to be sure, appears remote. But our present course is set in that direction and there are clear signs that significant progress is being made.

A Simplified Method of Producing Crossed Seed

For the present satisfactory increases in yield are obtained only by crossing inbreds in some fashion to produce an unstable combination for commercial use. What sort of recombination is best? The use of F_1 field corn hybrids from inbred lines seems to have found little favor. The relatively low yield of crossed seed at best and the uncertainty of the crop if adverse weather conditions prevail at the time of flowering are important factors limiting the usefulness of this scheme. Experience has shown also that the small size of the seed imposes a handicap on the hybrid plants at the critical seedling stage. Another disadvantage of using F_1 seed which has more recently become apparent is that, on account of its small size, it is not adapted to the present planting machinery. It has been considered possible too that, because of their genotypic uniformity, F_1 hybrids might be rather narrowly restricted in adaptability, but I am not aware of any experimental evidence on this point.

A type of combination which has found much more favor than the F_1 is the double cross. In this the yield of seed is high, the seed is similar in size to that of open-pollinated varieties and of good strength. High yields may be obtained from double-crossed seed, but the method of producing it is relatively complicated. Four parent inbred lines must be maintained and three separate crossing plots are essential in producing the ultimate product which goes to the corn grower.

The three-way cross is somewhat similar to the above. In it the F_1 hybrid between two inbred lines is pollinated with a third inbred stock and the resulting seed used for field production. It would be expected that this system would yield a more uniform product than the double cross and this greater uniformity might be an advantage. A general statement as to the relative yields that would be expected from double crosses and three-way combinations can not be made. The result with each system will depend upon the particular strains which enter into the crosses.

The prevailing practice, I believe, in making double crosses is to combine the four inbred strains in pairs and then to cross directly the two resulting hybrids for seed production. This system is one which is amenable to close control if, in the utilization of inbred corn for commercial purposes, it is found advisable to establish some kind of official seed certification. The F_1 hybrid plants used to produce the double-crossed seed are remarkably uniform in size and form and the ears borne upon them conform closely to one type. This should greatly facilitate identification of the breeding stocks in the field and the detection of any admixture or substitution. The price which is paid for this advantage, however, is constant repetition of the initial crosses between the four inbred lines to supply the parent stocks for the main seed plot. This step in the procedure is expensive on account of the labor involved in detasseling and the relatively low yields of F_1 seed obtained.

If we are prepared to forego this ready means of identifying the seed grower's stocks and uniformity of appearance in the seed ears another method of utilizing the double cross presents itself which is much simpler of management and should give, on theoretical grounds at least, an equally productive hybrid seed stock. The possibility has not been overlooked by Jones and Mangelsdorf (1926), and Kiesselbach (1928) has demonstrated its feasibility. It seems, however, to have escaped the general consideration which it would appear to merit.

According to the proposed method four inbred lines are crossed in pairs as in the double cross scheme. But the two respective hybrids instead of being used directly are allowed to come to equilibrium, a condition which, theoretically, is attained by random mating in the first generation after the cross. Thereafter the two lines may be maintained indefinitely in isolation as open-pollinated stocks. Hybrid seed for production purposes is obtained by crossing them. Two separated fields are all that the seed grower would require to perpetuate the parent lines and secure crossed seed.

This scheme is based upon the demonstrable genetic relationship that, providing there is no differential fertility or selective elimination, a random-bred family will produce in each generation the same types of gametes and in the same numerical proportions as the heterozygote from which it is derived. That is to say, the gametic array in F_2, F_3, F_4, etc., is identical with that in F_1. Consequently crosses between the two lines at any stage later than the F_1 would be expected to give the same result as a combination between the F_1 hybrids themselves.

This important principle, I believe, may be applied to advantage in the utilization of inbred lines of corn. Its chief merit lies in the fact that the parent stocks used to produce the main seed crop are not only productive but also self-perpetuating; they do not need to be renewed repeatedly by crossing the original inbred lines. One would expect that the hybrid produced by this method would yield as well as that obtained by crossing F_1 by F_1. The beautiful uniformity of the seed ears from the latter type of combination, however, would be lost.

It has been assumed in this discussion that the offspring of an F_1 hybrid reach a condition of equilibrium in the F_2 and that this would be maintained by random mating in subsequent generations. This is true only in the theoretically perfect case. Under field conditions variations in the productivity of the different genotypes would gradually modify the composition of the stock until a bal-

ance with the particular set of environmental conditions obtaining was reached. Approach to the new equilibrium would be accompanied, however, by a decrease in the proportion of those genes tending to lower productivity. Some increase in yield might be expected through automatic discrimination against the weaker segregates. It is rather hazardous to predict from general principles what effect such elimination would have on the ability of the stocks to produce a vigorous hybrid. The factor interactions involved are so complex and so little understood that the results of a given cross can be determined only by actually testing it. If the initial segregating populations were reasonably well adapted to the conditions under which the stocks were being grown, however, one would expect that the ability of the two random-bred lines to produce a vigorous hybrid would not be impaired.

TRADEMARKING SUPERIOR STOCKS

One of the most difficult problems to be faced in the utilization of inbred strains of corn is the maintenance of dependable supplies of crossbred seed. For these we shall probably have to look to skilled growers whose efforts are protected by some system of inspection and certification. The problem of certifying the seed of a grower who is using the familiar double-cross method should not be a difficult one. The two parent stocks he would use would be F_1 hybrids, and, consequently, uniform and distinctive in appearance. In view of their immediate relationship to the inbred parent, moreover, their lineage would be easy to follow.

What can be done, however, when the stocks crossed to produce commercial seed are the open-pollinated descendants of two pairs of inbred lines? In such material the variability in the parent strains may be so great that they can not be adequately described and hence not identified with certainty. This method of using inbreds is attractive in its simplicity and promising in yield but unless it can be made amenable to control in the trade it can scarcely be endorsed.

Could not such parent lines be trademarked in some way? Genetic investigations in corn have revealed a large number of clear-cut characters which are controlled in inheritance by single genes. The expression of many of these is dependent upon the germinal complex to such a high degree that the characters are identifiable over a wide range of environmental conditions. If the inbred lines entering into the recombination were made homozygous for some one distinctive character not occurring in present commercial varieties of corn, or found only rarely in them, identification of their descendants would be rendered easy and certain.

Obviously such a character would have to be chosen with discrimination. It should not detract from the vigor of the stock nor alter any feature which counts in the market. Indeed, the trade requirements would have to be closely observed. The latter are frequently arbitrary but varieties which do not conform to them are discounted. Many a worthy but novel product of the plant breeder's art has sought a blessing at the shrine of commerce only to be turned away because clothed in unfamiliar garb.

Undoubtedly within the range of monohybrid characters now known there are several which are suitable for the purpose in question. Three different genes for brown midrib, for example, have been discovered which have very superficial effects on the plant but which nevertheless impart a distinctive appearance. One of these, which was found at Madison in an inbred line of the Golden Glow variety, causes the formation during the later stages of development of the plant of a light brown pigment in the midrib of the leaf and to a lesser extent in the veins of the leaf sheath. It is a simple recessive to green midrib. There are numerous other characters available and new ones are being found continually.

A Gene Affecting Crossability

Many of the precautions which the breeder must take are a direct result of the fact that the corn plant is wind

pollinated. In the experimental breeding plot the young ear shoot is covered if mating is to be controlled, and larger cultures must be isolated if contamination by other corn would be avoided. The corn breeder has become accustomed to these measures and observes them as a matter of course. When stocks are released to the seed grower it can scarcely be hoped, however, that equally stringent precautions against admixture will be taken. It would be a decided advantage if a means were found which would obviate the necessity of isolating a strain of corn in order to keep it pure or at least to reduce the hazards of random pollination. From some results which Demerec (1929) has recently published it appears possible that this may be done by a genetic method.

Demerec has shown that a certain strain of pop-corn when used as the female parent in crosses with other strains is almost if not quite sterile. The pop variety is fully fertile with its own pollen, showing that there is no functional disturbance in the formation of female gametes; and its pollen is effective on all other strains tested. The pop line, however, sets few or no seeds when pollinated by other types. If one may judge from Demerec's results the variety is naturally almost as well protected against the pollen of other strains as is an ordinary corn plant with a bag placed over the young ear.

Demerec suggests that the female cross-sterility is probably due to the same factor which operates to produce a deficiency in the F_2 sugary ratio following matings between rice pop and sweet corns.[2] Low sugary ratios were first observed by Correns (1902); Emerson (1924) and Jones and Mangelsdorf (1926) have since studied them in considerable detail. It is essential to note here only that the findings of these later workers indicate that the peculiar behavior of the rice pop x sugary crosses is due

[2] Emerson, in a private communication to the author, states that at least some of the strains of rice pop-corn which he has found to give low sugary ratios do not show the cross-sterility described by Demerec. It seems doubtful, therefore, whether there is actually a relationship between the two phenomena.

to a single gene carried by the rice pop variety. This gene when present in an individual acts in some unknown manner to prevent fertilization by any male gamete not carrying the same factor.

A gene with the unique property of preventing cross pollination is worthy of the corn breeder's attention. He can possibly use it in the solution of one of his important problems, namely, the prevention of contamination of an improved stock by foreign pollen. Suppose, for example, that a double-cross combination of high merit is being distributed to seed growers. It is desired, of course, that the parent stocks held by the seed producer be kept pure. If ordinary varieties are being freely grown in the vicinity complete physical isolation may be impossible. But if the improved stock is not receptive to any pollen except its own, admixture will be automatically prevented even if other corn is grown in close proximity. It will be the fellow over the fence who will have to take care since the scheme works only in one direction.

It should not be difficult to breed such a physiologically isolated race. Demerec's strain is the initial source of the essential genetic factor. After outcrossing to this variety the type of the given inbred line into which it is desired to incorporate the factor could be regained quickly by repeated backcrossing. Of course, selection would be necessary during the procedure to maintain the cross-sterility gene in the stock, and the backcrosses would have to be made on the inbred line in each case since the reverse combination is not fruitful. I can see no disadvantages in such a scheme of preventing indiscriminate cross pollination, and it might be a valuable aid in the maintenance of purity.

Means of Detecting Contamination

Next to the actual prevention of cross pollination a ready way of detecting outcrosses when they occur would be helpful. The aleurone color situation in varieties of field corn suggests a possible way of accomplishing this.

Emerson's (1918) thoroughgoing studies have shown that in order that pigment be produced in the aleurone three dominant genes, *A*, *C* and *R*, must be present together with *i* in homozygous condition. If any one or more of the three factors *A*, *C* and *R* is replaced by its respective allelomorph the aleurone forms no pigment. As is well known, all the field corn varieties have colorless aleurone. According to Emerson's survey this is due in large part to their being *rr*. In many cases *A* and *C* are present so that but one of the elements is lacking to produce color.

Now if it were desired to put an improved strain in condition so that crosses with the standard varieties with which it would be likely to become contaminated in the field could be detected the improved stock could be made up as a *c*-tester. That is, with respect to the aleurone color factors it would be *AA cc RR*. The seed, of course, would be colorless as before, so that this requirement for field corn would still be met. In such a stock grown where it was exposed to cross pollination by ordinary varieties any outcrossed seeds would be directly revealed by the color produced in the aleurone. Color in the seed would serve as a badge of mongrelism. Any considerable proportion of colored kernels would stamp the material as unfit for seed. If only a few were present the stock could be rogued in the ear. Breeding the stock as an *a*-tester would be an equally effective means of detecting outcrosses. Such a strain, however, would have white cobs (if the gene, *P*, for pericarp color is present the cobs would be brown) and this might be undesirable.

Objection might be raised to this scheme on the grounds that the planting of such seed in districts where considerable corn of ordinary varieties is grown would result through interfield crossing in the production of some colored seeds. Where corn is being grown for the market and sold on grade such contamination might be important. If the corn is consumed on the farms where it is produced, on the other hand, this outcrossing would not detract from the value of the crop.

A Criterion of Segregation[3]

One of the chief problems which may be foreseen now in the wide use of crossbred corn will be the tendency of growers to save seed from the hybrid crop. The immediate decrease in yield which will result from this practice may go far toward nullifying the advantage to be gained from using hybrid corn. The success of the whole scheme as an agricultural proposition turns largely on the degree to which farmers will act on the principle that maximum yields are to be obtained only from crossed seed produced by two parent stocks bred specifically for that purpose. There must be a clear recognition of the fact on the part of the general grower that the corn he raises, however attractive in appearance it may be, can not duplicate the yield of the parent crop.

The difficulties which stand in the way of the general acceptance of this practice are probably underestimated at the present time. Much can be accomplished by demonstrating the loss in yield occasioned by any departure from the correct procedure. I am inclined to believe, however, that the wide adoption of a sound practice in producing and growing crossed corn could be greatly accelerated if the stocks are bred in such a way that the difference in character of hybrid seed and seed from plants one or more generations removed would be apparent in the seed itself. The farmer, generally speaking, is not in a position to detect or measure the possibly small but nevertheless significant decreases in yield which will result from the use of random pollinated seed from a crossbred crop. A visible difference in the appearance of such seed, on the other hand, would go far in establishing the conviction in the grower's mind that the essential characters of his stock had altered.

There are two pairs of monohybrid characters expressed in the seed occurring in standard commercial varieties which might be used for the purpose of demonstrating segregation. The first of these is yellow and

[3] This section was not presented at the Des Moines meeting.

white endosperm. In recent years white corn has so declined in favor, however, that such a combination might meet resistance on account of the 25 per cent. of white seeds produced on crossbred plants. So far as the difference in color is associated with a disparity in feeding value the prejudice is likely to persist, and tests do show a higher content of vitamin A in yellow corn. The difference, moreover, appears to be directly associated with the yellow color. This objection could be met in part at least by using the dominant white-cap character which in yellow corn does not entirely suppress the development of the pigment.[4] The White-Cap Yellow Dent and Silver King varieties are familiar sources of the white-cap gene.

A crossbred stock which would be uniformly white-capped in appearance and which would segregate white-capped yellow and yellow seeds in the next generation could be bred without difficulty. If hybrid seed were being produced by the double-cross method the two parent lines combined in one of the initial hybrids could be made homozygous for white capping and yellow endosperm and the other two lines homozygous for yellow endosperm alone by repeated backcrossing and selection. In the second cross all the seeds would be white capped, their uniformity in this respect marking them as suitable for seed. The crop which they would produce, however, would be mixed in color, white-cap yellow and yellow, symbolizing the phenomenon of genetic segregation by which the yielding capacity of the stock is simultaneously lowered.

PHYSIOLOGICAL ISOLATION BY MEANS OF TRANSLOCATIONS

I wish to mention briefly one other phenomenon which genetic investigations have revealed that may prove of interest in corn breeding. Like the cross-sterility factor mentioned earlier it provides a means of isolating a variety from other types of corn by physiological means.

[4] Russell (*Journ. Nutrition*, 2: 265–269) has recently shown that white-capped yellow dent corn contains vitamin A but in a somewhat smaller amount than yellow corn.

I am forewarned, however, that it is far too dangerous a mechanism to be allowed to get beyond the confines of a geneticist's workshop. Dire consequences of its release have been predicted. Its effectiveness for the purpose in mind, namely, of preventing the degradation of a superior variety through natural crossing by less desirable stocks, is admitted, but I am asked if this can not be attained by less hazardous means.

As will have been surmised the scheme has already been the butt of some abuse. Nevertheless I shall venture to discuss the phenomenon here not with the thought, however, that further criticism will be escaped but because of the unique property it confers upon a stock and of possibility that it may sometime prove useful.

I have at Madison a race of corn which, aside from the handicap of having arisen from the debauched stock with which a geneticist deals, is in no essential way different in appearance from normal representatives of the species. The strain in question grows vigorously and produces a full and well-developed ear. But when it is crossed with other lines of corn the offspring in their turn produce only 50 per cent. functional pollen and the ears bear one half the normal number of seeds. The hybrids, in brief, are semi-sterile. Genetic analysis indicates that the abortion is caused by an alteration in two of the ten chromosomes in the normal corn complement. Either there has been an interchange of segments between the two chromosomes or a piece has become detached from one and affixed to the other whole chromosome (Brink and Burnham, 1929). The abortion is due to the fact that one half the pollen grains and ovules receive unbalanced sets of genetic factors. The pure-breeding line which produces semi-sterile plants when crossed with other strains of corn is called x-normal. It is homozygous for the translocation. On the hypothesis that a single translocation is responsible for the peculiar behavior of the x-normal stock a particular group of linked genes from one chromosome is transferred to another chromosome

and assorts with it. No genes have undergone mutation or are missing; the change is entirely one of position in the chromosomes. Since the x-normal condition is independent of the kinds of genes present it can be bred into any strain of corn.

The original case of semi-sterility in corn was discovered in 1925. In 1927 another one was found. By combining the two it should be possible to produce a fully fertile race which would give hybrids with normal corn showing 75 per cent. sterility. Two additional cases of partial sterility have been discovered since, but it is not known yet whether they involve the same type of change. It is fully expected, however, that further cases of semi-sterility will be discovered. A third translocation would afford the means of breeding a fully fertile stock which in crosses with ordinary corn would cause 87.5 per cent. sterility, and so the series runs. With five translocations a race could be produced which, while normal in appearance and productivity, would give hybrids almost wholly sterile with the varieties of corn now being grown. Remaining identical in type it would behave like a distinct species in its fertility relations.

The production of a fertile stock containing more than four or five translocations offers some difficulties on account of the sterility involved but possibly it could be accomplished. Such a race would be physiologically isolated from present-day types of corn. By this is meant that the two groups would not interbreed readily. Crossing could occur but the hybrids would leave few or no offspring.

As a means of guarding an improved strain against admixture with ordinary varieties to whose pollen it would be exposed translocations would undoubtedly be effective. It must be granted, however, that the method has drawbacks. The most serious of these is that the protection is paid for by reduced yield. Every outcrossed seed will produce a barren plant. Then, too, whenever the pollen of this hypothetical stock gets into

the corn field of some one who is still faithful to an old variety it will sterilize every embryo into whose composition it enters.

The method proposed of accomplishing physiological isolation through the use of the cross-sterility gene found in pop-corn may prove just as efficient and is certainly less insidious in its action. That factor operates to prevent hybridization. It stops the invader at the door, so to speak. The translocation method admits the stranger but later dismembers him.

Perhaps a mechanism charged with such subversive properties is best kept under guard for the present, at least. If the need should arise in corn breeding, however, for some virulent means of protecting an improved product, it is at hand.

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INHERITANCE IN A MOUSE SPECIES CROSS

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A STUDY of inheritance in a mouse species cross has thrown light upon a number of phases of the general problem. Among the more important of these are litter size, birth weights, growth, Mendelian ratios, sex ratios and size inheritance. The present notes touch upon the latter three.

The forms involved in this cross were a small, light-colored, white-bellied Asiatic mouse, *Mus bactrianus* (formerly often designated as *Mus wagneri*), and the Little inbred strain of dilute brown, non-agouti *Mus musculus*. The original animals of our *Mus bactrianus* stock were captured near Peiping, China, and brought to the United States by Dr. Sheo Nan Cheer in 1926. Dr. G. M. Allen has identified specimens of this stock as intermediate between the Gobi Desert race, *Mus bactrianus gansuensis* Satunin, and the race inhabiting Western China, *Mus bactrianus tantillus* Allen (1927), although on the whole nearer the former. *Mus bactrianus* attains less than half the adult weight of *Mus musculus* and, in addition to size, differs in many other respects, such as color, the possession of fewer caudal vertebrae, peculiarities in the shape of the skull and a relatively shorter tail.

Bactrianus males cross readily with *musculus* females as noted by Little (1927), and the hybrids of both sexes are fertile. The reciprocal cross has also been made although, perhaps because of the discrepancy in size, it takes place less readily. The F_1 hybrids from both types of matings are identical in appearance. In the production of the back-cross generation all F_1 animals employed were from *musculus* females.

Since our intense black agouti *bactrianus* are triple dominants (DBA^w), and the dilute brown non-agouti *musculus* are triple recessives (dba), the F_1 hybrids

mated to the recessive parent should produce offspring of eight color classes in equal numbers if the genes of the two species are allelomorphic and show no tendency towards the formation of association systems of chromosomes as Gates (1926) found in the Japanese Waltzer (probably derived from *Mus bactrianus*)—*Mus musculus* cross. A summary of the total back-cross data, given in Table I, shows approximate equality in the eight classes with no significant excess of the two parental types.

TABLE I
TOTAL BACK-CROSS GENERATION

	BlkAg (DBAw)	dBlkAg (dBAw)	BrAg (DbAw)	Black (DBa)	Brown (Dba)	dBlk (dBa)	dBrAg (dbAw)	dBr (dba)	
Observed	86	96	95	89	97	72	97	108	740
Expected	92.5 ± 6.1	92.5 ± 6.1	92.5 ± 6.1	92.5 ± 6.1	92.5 ± 6.1	92.5 ± 6.1	92.5 ± 6.1	92.5 ± 6.1	740

It is well known that a phenomenon often attending hybridization is a distortion in the sex ratio. King (1911), among others, has observed this in interracial crosses in rats. Such distortions, however, have been usually recorded in the F_1 generation only. Our data in Table II show the first hybrid generation to be unaffected, while only the back-cross exhibits indications of a heightened sex ratio.

TABLE II
SEX RATIOS

Mating	No. of males	No. of females	Ratio (No. ♂'s to 100 ♀'s)
bactrianus ♀ × *bactrianus* ♂	178	171	104.9 ± 7.57
musculus ♀ × *musculus* ♂	586	545	107.52 ± 4.35
musculus ♀ × *bactrianus* ♂	104	101	102.97 ± 9.72
bactrianus ♀ × *musculus* ♂	29	31	93.55 ± 16.36
musculus ♀ × $\frac{musculus}{bactrianus}$ ♂	203	164	123.78 ± 8.76
$\frac{musculus}{bactrianus}$ ♀ × *musculus* ♂	304	258	117.83 ± 6.76
Combined back-cross generation	507	422	120.14 ± 5.34

In the study of adult size inheritance, ten quantitative indices were chosen. These were: body length, tail

length, relative tail length, number of caudal vertebrae, adult weight, skull length (the distance from the highest point of the foramen magnum to the most anterior surface of the incisor teeth), skull width, humerus length, femur length and tibia length. No animals of a lesser age than 180 days were included in the tabulations of quantitative characters (with the exception of the number of caudal vertebrae), so it is probable that the characters selected had attained their maximum development.

In body length, tail length, relative tail length, number of caudal vertebrae and adult weight, the F_1 animals were intermediate between the two parental species. The back-cross animals were likewise, for the most part, intermediate between the F_1 and the *musculus* parents. In skeletal measurements, however, both F_1 and back-cross mice generally equaled or surpassed the larger parent race.

As would be expected from the degree of inbreeding which they have undergone, the *musculus* mice were less variable than the *bactrianus*. The F_1's were intermediate in variability, while the back-cross animals were in general more variable than the first hybrid generation.

In order to find out whether there has been any tendency for the larger size of the parent, recessive for three qualitative characters, to remain associated with the recessive characters, animals of the back-cross matings, treating each type separately, have been divided into dominants and recessives, considering only one pair of allelomorphs at a time. Thus all males of the back-cross mating *musculus* ♀ $\times \frac{musculus}{bactrianus}$ ♂ carrying the dominant agouti factor A^w were compared with all non-agouti males with the gene a. Similarly the blacks (B) and browns (b), and the intense (D) and dilutes (d) were compared. This same procedure was carried out for the females and for both sexes of the reciprocal back-cross mating. Thus the ten quantitative indices, when considered in conjunction with the three factor pairs and the two sexes, make available for comparison sixty pairs of values (dominant *vs.* recessive) in each type of back-

cross mating. These comparisons are summarized in Table III.

TABLE III

QUANTITATIVE INDICES IN RELATION TO THE DOMINANT AND RECESSIVE MEMBERS OF FACTOR PAIRS

Back-cross mating	Recessives greater	Dominants greater	No difference
musculus ♀ × F_1 ♂	38	19	3
F_1 ♀ × *musculus* ♂	44	12	4
Total back-cross	82	31	7

From this table it is apparent that there has been a general tendency for those animals showing recessive qualitative characters to exceed their dominant allelomorphs in the quantitative indices investigated. Since this tendency seemed to be manifested in all three allelomorphic pairs a comparison of the extracted triple reces-

TABLE IV

EXTRACTED PARENTAL TYPES FROM BACK-CROSS GENERATION

Character	No.	♂ (DBA^w)	No.	♂ (dba)	Difference
Body length (mm)	16	92.1 ± .58	19	95.1 ± .66	3.0 ± .88
Tail length (mm)	15	87.3 ± 1.10	18	91.5 ± .82	4.2 ± 1.37
Tail length (percentage)	15	48.5 ± .27	18	49.1 ± .17	.6 ± .32
Caudal vertebrae	19	23.8 ± .10	25	23.8 ± .08	.0 ± .13
Adult weight (gms)	14	27.4 ± .76	19	29.1 ± .83	1.7 ± 1.13
Skull length (mm)	16	21.83 ± .087	19	22.14 ± .068	.31 ± .110
Skull width (mm)	16	4.15 ± .026	20	4.12 ± .023	−.03 ± .035
Humerus lgth. (mm)	16	11.40 ± .063	20	11.72 ± .073	.32 ± .096
Femur length (mm)	16	14.66 ± .115	17	14.93 ± .097	.27 ± .150
Tibia length (mm)	16	16.79 ± .122	20	16.92 ± .168	.13 ± .208

Character	No.	♀ (DBA^w)	No.	♀ (dba)	Difference
Body length (mm)	15	91.6 ± .69	27	94.2 ± .49	2.6 ± .85
Tail length (mm)	14	87.2 ± .72	23	87.8 ± .70	.6 ± 1.00
Tail length (percentage)	14	48.7 ± .17	23	48.1 ± .27	−.6 ± .32
Caudal vertebrae	19	23.8 ± .10	22	24.1 ± .06	.3 ± .12
Adult weight (gms)	15	23.1 ± .54	25	26.7 ± .67	3.6 ± .86
Skull length (mm)	14	22.02 ± .077	25	21.99 ± .060	−.03 ± .097
Skull width (mm)	15	4.15 ± .022	26	4.11 ± .018	−.04 ± .028
Humerus lgth. (mm)	13	11.05 ± .063	26	11.28 ± .041	.23 ± .075
Femur length (mm)	11	14.60 ± .149	21	14.72 ± .067	.12 ± .163
Tibia length (mm)	14	16.48 ± .150	25	16.79 ± .079	.31 ± .169

sives with the triple dominants should be more effective in detecting the presence of linkage. These comparisons are given in Table IV.

These differences, while in many cases not significant statistically, are certainly strongly indicative of linkage between qualitative and quantitative characters although such a situation has never been conclusively demonstrated in laboratory mammals. Castle (1929), for example, after an extensive investigation, could find no indications of such a relationship in rabbits.

The study of size inheritance in this mouse interspecific cross has shown it to be generally of the blending type with an increase in variability of the back-cross over the F_1 generation. In addition, there is strong indication that certain genetic factors productive of a large final size are found in the three chromosomes of the *Mus musculus* parent carrying the genes d, b and a. In order to determine this conclusively further work has been undertaken. The growth curves present certain indications that more factors—or factors of greater potency—influencing the adult weight are found on the chromosomes with a and b than with d. A survey of the whole makes it appear probable that size in mice is due to a number of chromosomal genes, most, but not all, of which are general in their action. The differences in behavior of the various skeletal measurements suggest the existence of certain local factors.

A more complete account of this investigation will be published elsewhere.

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SOME OBSERVATIONS ON THE BIOLOGY OF THE MALE *DROSOPHILA MELANOGASTER*

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AN experiment[1] was undertaken to test the fecundity of the male of *Drosophila melanogaster*. Wild-type males were mated to females of the mutant bar type. As soon as the mating was completed the female was put into a separate bottle. The males were mated as many times per day as they would mate. After they were three days old they would mate from two to five times per day until toward the close of the fertile period. The disturbance caused by separating the flies and putting in another virgin female with the male prevented the male from mating as many times as he might have done, as shown by the following experiment.

A male, eighteen days old, was put into a bottle with ten virgin females seven days old. He mated with seven of them between the hours 8.07 A. M. and 12.24 M. He was then removed and put with other virgin females in the afternoon of the same day. He mated with two of them. His average time of mating was 20.8 minutes. The last five matings were kept and all were fertile, averaging 95 offspring.

No male used in the experiment was mated before the second day following his emergence from the pupa case. The female of each mating was kept in a separate bottle and counts made of the number of her offspring. For the most part, counts were made every day. Since the flies were removed from the bottles while young, a second generation did not get started. However, the experiment was so planned that if flies of the second gen-

[1] The work was done at the William G. Kerckhoff Laboratories of the Biological Sciences, California Institute of Technology, Pasadena. I wish to express my appreciation to Dr. T. H. Morgan for placing at my disposal every convenience necessary.

eration came on they could be recognized. Wild males mated to bar eyed females, the types used in the experiment, give bar eyed sons and heterozygous bar eyed daughters. The second generation, if it came on, would consist of bar eyed and normal eyed males and bar eyed and heterozygous bar eyed females. The appearance of homozygous bar eyed females would be evidence of a second generation.

TABLE I

RECORDS OF MATINGS OF MALES OF *Drosophila melanogaster*. AGE GIVEN IN DAYS

No. of male	Age at first mating	Age at last mating	Age at last fertile mating	Number of fertile matings	Serial number of last fertile mating	Total number of matings	Age at death	Total number of offspring	Avg. no. offspring per day of fertile period
3	2	69	51	84	104	121	72	12968	265
4	2	53	26	61	66	92	60	7807	312
5	20					49	killed	sterile	
6	34	63	63	39	44	44	66	7638	263
7	2	48	38	71	77	85	53	11819	309
8	2	48	35	57	65	77	killed 52	10195	270
9	57	95	91	39	39	39	killed 99	7805	230
10	2	43	43	66	71	71	43	14070	335
11	2	43	34	63	65	73	killed 43	11889	350
12	2	.....	22	43	45	64	killed	7368	368
								Percentage of males 49.01	

The total number of offspring from one male ranged from 7,368 to 14,070. From the table it may be seen that males No. 6 and No. 9, which gave offspring below ten thousand, were not mated until comparatively old. No. 4 and No. 12 also gave the fewer number of offspring. A note made when No. 4 was mated states that "it is a dis-

tinctly smaller male than the average." No. 12 male was, perhaps, also a small male, as he was taken from a stock culture which was old and the food about used up. Loeb and Northrop (1916) produced sterility in males by raising the flies on sterile food. One might expect, then, that if a fly is undernourished its fecundity would be below the average, as was the case with No. 12 male.

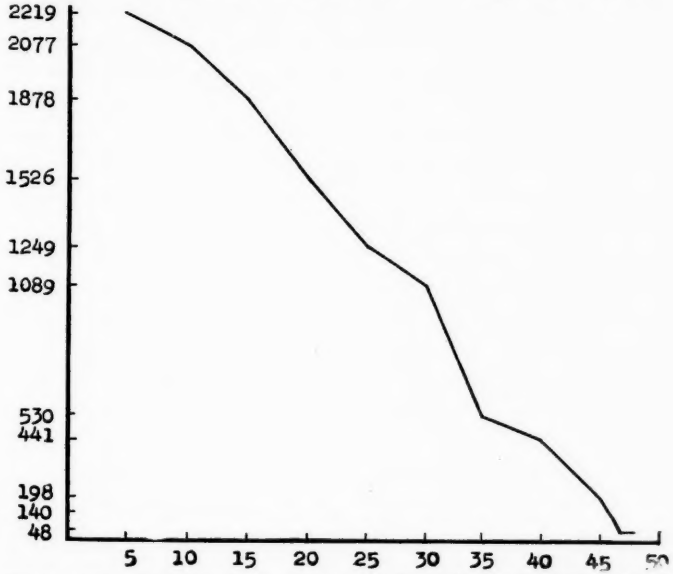

FIG. 1. Curve of productivity of the male *Drosophila melanogaster*, based upon the average number of offspring produced in five-day periods.

Fig. 1 shows that reproduction is highest for the first five days with an almost regular decline to thirty days. At this time there is a sharper decline in the productivity. The average fertile period for the males was 32 days, and at this time there is a noticeable diminution in the number of offspring of all males. The curve is based upon a five-day period in order to reduce the effect of variables. The known variables were the different number of times per day that a male was mated, the occurrence of sterile females and, in one case, a sex linked

lethal which killed off half the male offspring. The total number of offspring for the five-day period was divided by the number of males reproducing for that period. From the fortieth day on only one male produced offspring.

The number of offspring is not a complete index of the number of sperm produced by the male. Huettner (1927) says, "One can usually count a dozen or more sperms in the anterior region of most eggs." He figures a section of an egg in which he says, "Hundreds of sperm could be observed." He concludes that such extreme polyspermy as the last prevents the development of the egg, but several sperm may not be unusual or give abnormal results.

The males used in this experiment were dissected and sperm were found to be present in the testes of all of them. Some were killed as soon as the fertile period was known to be over; others were kept until they died and then dissected. The testes of some were sectioned and showed mitotic figures in the small spermatogonial portion, proving that sperm were still in the process of formation.

The males continued to mate after their fertile period was over. For example, No. 3 male mated 121 times; the last fertile mating was the 104th. He continued to mate once per day, with the exception of one day, until three days before his death. The mating time was kept for this male to see if there was any correlation between the mating time and the number of offspring. No such correlation was found. The average time of mating for the fertile period was 24.4 minutes; for the seventeen matings following this period it was 30.6 minutes. There were sterile matings at the end of the fertile period of all males except No. 6 and No. 9, which were old at the time of the first mating, and of No. 10 which was killed for sectioning. Sterility is not due to the lack of sperm in the testes. This fact was also shown by No. 5 male. He

was mated 49 times and produced no offspring. Dissection showed that the testes were full of sperm. There was perhaps some defect in the ejaculatory apparatus of such nature that no sperm could pass out.

An experiment was devised to test the possibility that this post-fertile period of sterility might be due to the absence of some activating fluid secreted during the earlier period and not secreted in the later period. Nonidez (1920) had shown that a female will mate with another male while there are still sperm from a first mating in the spermathecae. The sperm of the first mating become functional after the sperm of the second mating have been used, or nearly used up. This work of Nonidez was verified by Nachtsheim (1927). Of the sixty-four matings of No. 12 male 46 were fertile and 18 sterile. There was only one individual, a male, produced in the 46th or last fertile mating. The fact that the matings were sterile was not recognized until ten days after the matings had taken place, at which time offspring were due. Immediately all the females from the 46th mating on to the 64th which had mated to No. 12 male were mated to bar males. These matings to the bar males took place in the females Nos. 46 to 58 ten and eleven days after the mating to No. 12 male; in the females Nos. 60 and 61 on the day following the mating to No. 12 male, and in Nos. 59, 62, 63 and 64 three days after the first mating. The sperm of the mating to the bar male would first be used and then, if there were any functional sperm from the mating to No. 12 male, there should appear heterozygous bar eyed females. The offspring were removed every day until no more were produced. The results were completely negative since no offspring having heterozygous bar eyes were found in any of the bottles. Hence it seems justifiable to conclude that no sperm entered the sperm receptacles of the female during the mating with No. 12 male.

This experiment was also tried by reversing the order in which the males mated. Bar females that had mated

to bar males were mated to a wild-type male that had become sterile by means of numerous matings. Offspring from the mating with the wild-type male would appear among the first produced by the bar female. No such offspring were found. Virgin females mated to this male were dissected and no sperm could be found in the ventral receptacles or in the spermathecae. Since this sterile period occurs in all males mated as frequently as they would mate, it is probable that sterility is caused by the enfeeblement of the ejaculatory sac, described by Nonidez (1920) as "the mechanism that drives the sperm through the ejaculatory duct."

No. 9 male deserves special notice. He was not mated until he was 57 days old. For the first six days he mated twice per day and on the second day three times; after that he would mate but once per day, toward the end often skipping one or two days. His fertile period was 31 days, only one day below the average for all the males. He lived 99 days and at the last got stuck in some soft food from which he was too feeble to extricate himself.

These data also give the number of reversions of bar to normal in the F_1 generation when a normal male is mated to a homozygous bar female. In 78,587 flies there were 64 reversions, 22 female and 42 male, which gives one reversion in 1,228 flies.

Summary

A vigorous male *Drosophila melanogaster* should produce from 10,000 to 14,000 offspring; a smaller, less vigorous male should produce about 7,500.

The average fertile period of males is about thirty-two days. Following the fertile period is a sterile period which varied in time in the males used from 21 to 34 days.

Sperm were observed in the testes of all males after their fertile period was over.

Mitotic figures were found in sections of the testes of

males after they had come to the period of sterility, showing that sperm were still being formed.

Tests were made to find out whether the failure of sperm from old males might not be due to a deficiency of some fluid from the accessory glands. The results indicated that this was not the case. No sperm were found in the sperm receptacles of females that had mated to these males during their sterile period, excepting, of course, the cases of double matings. Therefore, the impotency of the old males must be due to a loss of function in the ejaculatory apparatus.

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AN ATTEMPT TO INDUCE MUTATION IN *DROSOPHILA MELANOGASTER* BY MEANS OF SUPERSONIC VIBRATIONS

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Introduction

The successful attempt of Muller (1928), Stadtler (1928) and others to produce mutations by means of X-rays, and the similar success of Stadtler (1928) and Hanson and Heys (1928) with radium, along with the further analysis of the action of radium by Hanson and Heys (1929) combined with the negative results of Mann (1923), Altenburg (1928), Muller (1928) and others with diverse chemical and physical agents, have suggested the conclusion that perhaps the electron is unique in its action as the cause of variation in the higher organisms. It remains, then, a matter of some importance to determine the effects of other agents in this respect.

The remarkable physical, chemical and biological effects of sound waves of high frequency make it a matter of interest to determine whether such vibrations may be effective in the production of mutations. To what extent positive results with supersonic vibrations would affect evolutionary theory would depend upon the occurrence and intensity of such vibrations in nature.

This paper is the report of an attempt to induce mutation by the use of these vibrations. The results are inconclusive. The manner of production of supersonic vibrations and some of their effects have been described by Wood and Loomis (1927) and by Harvey, Harvey and Loomis (1928).

Treatment and Results

A chamber in which to expose the flies was constructed by sealing end-on pieces of glass tubing of different

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diameter. The open end of the chamber had a diameter equal to that of the vials (25 mm) in which the flies were kept. The end of the smaller tubing (8-mm diameter) was closed by fusion. It was thought that by constraining the flies in a small space it would insure more uniform exposure for all. The exposure tube or chamber was immersed in the oil bath of the oscillating quartz plate, and firmly held so that its closed end was about 5 mm from the quartz plate. Each set of flies was transferred from the vials into the exposure tube and gently shaken down to the bottom. As soon as oscillation began the flies were stimulated and endeavored to climb up the sides of the narrow tube, but fell back because of the violent vibration of the walls. After a few such attempts they became inactive in a heap at the bottom.

In the specially made glass chamber, about a thousand cut forked Bar males, in lots of approximately 50 at a time, were subjected to 285,000 vibrations per second. Flies subjected to the treatment for 60 seconds were overcome and failed to revive. With a treatment of 40 seconds, the flies were likewise overcome; many apparently revived with difficulty, but before mating all had died. All flies which were mated had been subjected to 285 kilocycles for about 25 seconds. The treatment was given at Tuxedo Park, New York. Matings were made several days later at Cleveland, Ohio, during the first week of September, 1928.

The treated cut forked Bar males were mated to 429 virgin double-yellow females with attached X-chromosomes. These flies had been closely inbred for about eight generations previous to the beginning of the experiment. In this type of mating the daughters are like their mothers and the sons are like their fathers in regard to sex-linked traits. Each son receives his X-chromosome from his father, so that any visible mutation, dominant or recessive, which might occur in the X-chromosome of the germ-cells of the treated fathers may be detected in the sons of the first generation. Recessive mutations occurring in the autosomes could be detected only in the

second generation, and such mutations were not looked for. Dominant mutations occurring in any of the autosomes, as in other types of matings, could be detected in the first generation offspring. Lethals can not be detected by this type of mating.

Matings were made in 3 x 1 inch glass vials on banana agar food. The progeny were raised at about 25° C. Several times during the course of the experiment the temperature rose for short intervals to about 28° C. but never fell below 25° C. Of the 429 matings, 302 gave progeny as follows:

12,189	double-yellow females
13,598	cut forked Bar males
290	superfemales
30	heterozygous Bar females
28	yellow not-Bar males
26,135	total

The number of superfemales compares favorably with the number obtained by Dobzhansky (1928) in the progeny of similar females raised at 24.5° C. The heterozygous Bar females and the yellow not-Bar males indicate disjunction of the attached X-chromosomes of the mother. Their rate of occurrence is about five times greater than that obtained by Dobzhansky. The difference may arise from the fact that in this experiment the double-yellow mothers were collected from cultures kept at room temperature, about 23° to 28° C. The following distribution of the superfemales in the progeny of the 302 fertile matings may be of interest:

184	gave	0	superfemales
57	"	1	"
25	"	2	"
18	"	3	"
11	"	4	"
3	"	6	"
1	"	8	"
1	"	9	"
1	"	11	"
1	"	19	"

Among the 26,135 first generation progeny there was an occurrence of abnormalities arranged according to the part of the body affected and by sex as follows:

	Females	Males
Legs (misplaced anterior pair)	1	0
Wings (wrinkled, crumpled or withered)	7	5
Short blunt scutellar bristles	6	0
Black abdominal "tumors"	4	0
Abnormal abdomen (mainly incomplete sclerites)	7	15
Small body	3	4
Mottled eye	0	5

Of these fifty-seven abnormalities, only the mottled eye was definitely shown to be hereditary. The other 52 representing perhaps ten different types either gave no progeny or the progeny were normal. They were found among the progeny of forty different sets of parents. This is an occurrence of non-inheritable abnormalities of about 13 per cent. on the basis of families and of about 1 in 500 flies or 0.002 per cent. on the basis of the total number of flies examined.

There are very meager data in the literature on the percentage rate of occurrence of these non-inheritable abnormalities. Mann (1923a) found that among 7,292 flies from alcoholized parents there were no abnormalities. In her experiments with arsenic there was found a somatic abnormality—a characteristic stunting of growth—which occurred on the average about once in 24 flies. The total number of non-inheritable abnormalities for the 45,497 control flies is not given (Mann 1923b). Our control flies, which were supplied from an experiment performed at about the same time as our own by Mrs. Roselle K. Hersh, and which she has kindly allowed us to use for the present purpose, are also somewhat inadequate in this respect. In this experiment, in the progeny of double-yellow females by cut forked Bar males, the primary purpose was a search for possible mutations at the Bar locus. Other parts of the body than the eye were examined only secondarily. No abnormalities were recorded in a total of 7,182 females and 7,259 males. The general impression which one gets of the total situation is that

the rate of occurrence of abnormalities in the progeny of the treated males seems an unusually high one. A factor other than the treatment in which the control and experimental flies differed, and which must be taken into consideration in estimating the effect of the treatment, arises from the fact that the treated males were mated several days after the treatment. We have then the possible effect of stale spermatozoa. The flies were raised in several broods by transferring the parents every 7 days to fresh bottles of food. Of the 40 families which gave the 52 non-inherited abnormalities, 29 gave no third broods; 6 gave no second broods. The abnormalities were obtained from 27 families in the first brood, 9 in the second brood and 1 in the third brood. There were 2 cases in which abnormalities occurred in both first and second broods, and one case in which both the first and third broods of the same family yielded abnormalities. This falling off in rate of occurrence of the abnormalities with the broods can not, however, differentiate between a possible effect of the treatment and a possible effect of old spermatozoa. The attempt was made to examine the later broods as carefully as the first.

In regard to mutations at the Bar locus, among the 7,259 males of the control flies there were found 14 with exceptionally large eyes and 9 in which the eyes were exceptionally small. These all gave progeny in which the average size of the eyes fell well within the normal range of variability for Bar, although no facet counts were made. In the 13,598 Bar-eyed progeny of the treated flies occurred 16 with exceptionally large eyes and 12 with exceptionally small eyes. These likewise gave progeny within the normal range of variability, so that the males tested were apparently merely extreme fluctuations of Bar. In the type of mating used, if a reversion to full had occurred it would have been indicative of a return to full unaccompanied by crossing-over (see Sturtevant, 1925). No such reversions were found by us, but they have since been reported by Hanson (1928). In the con-

trol and experimental flies, then, a total of 20,857 males gave no readily detectable mutations at the Bar locus.

Occurrence of mottled eye.—In the progeny of three different males appeared five males with mottled eyes, which were all obviously of the same type. Their occurrence in the males only suggested sex-linkage. However, when the males were bred to double-yellow sisters, the F_1 flies in each case were normal. These were inbred and in the F_2 the mottling was again recovered in a few males and also females, but only in the descendants of 2 of the 5 original males. The character has since been carried along in the same type of mating with a greater or smaller number occurring in each generation. In comparison with the non-inheritable abnormalities, we might say that mottled eye is barely inherited, if it is permissible so to describe it. Such a statement emphasizes that the non-inheritable abnormalities at present enjoy a rather unsatisfactory status. Some of them at least are almost certainly inherited in the sense that flies which show them have a different genetic constitution from flies which do not show them, raised in the same environment. To speak of such abnormalities in the usual way as accidents or as by-products is describing things merely teleologically.

Mottled eye is definitely hereditary in the sense that it recurs in the progeny of mottled flies, but in small numbers. A pure breeding stock has not been recovered as yet, but our experience with it up to the present time indicates that the mutation is perhaps of the ever-sporting kind.

In the Bar-eyed males the dark mottling is seen near the line of division between the dorsal and ventral lobes, with the tendency for the dorsal lobe to be more affected perhaps than the ventral. In the full-eyed double-yellow females the mottling may vary from a single small intensely dark speck along the dorsal edge of the eye to dark mottling over the entire eye. Very commonly only the dorsal half approximately of the eye is affected, sug-

gestive of a dorsoventral gradient. The smaller the affected areas, the darker is the mottling, indicating perhaps a constant relation between the areas involved and the amount of pigment. Among the millions of *Drosophila melanogaster* which have been examined, the occurrence of a mottled eye has been reported but twice before, and these were both found among the progeny of X-radiated flies, one by Muller (1928) and one by Weinstein (1928). However, these three separate occurrences, Muller's, Weinstein's and our own, seem to rest upon different genetic bases.

The occurrence of such a rare character as mottled eye in the progeny of 3 of the treated males indicates an overwhelming probability that the same underlying cause is involved. Although no mottled eye was found in the 14,441 control flies, yet the cause may not be referred to the treatment, because of the very close genetic relationship existing between the 3 males, with the consequent possibility that the mutation was already there, and that its detection merely concurred with the treatment. The aberrant manner of its inheritance, along with the extreme rarity of mottled eye, is also a hindrance to looking upon the treatment as its cause. Our experience indicates the great desirability of further testing the effect of supersonic vibrations in producing mutations, with perhaps the use of greater intensities than that used in this experiment.

SUMMARY

From cut forked Bar males which had been subjected for about 25 seconds to sound waves with a frequency of 285,000 vibrations per second, and then mated to double-yellow females, there were raised a total of 26,135 flies. Among these were found 52 abnormalities of about 10 different types that were not inherited. There was also found a mottled eye mutation, which is "ever-sporting." No mutations or reversions were found at the Bar locus among a total of 20,857 males from the control and treated flies. The evidence is inconclusive that the abnor-

malities and the mutations were caused by the treatment, but it is definitely shown that supersonic vibrations of the intensity used and under the conditions of the experiment do not produce effects comparable to those produced by X-rays and radium.

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SHORTER ARTICLES AND DISCUSSION

THE THEORY OF DOMINANCE

In the past the phenomenon of dominance has largely been taken for granted, or accounted for on a crude hypothesis of genic loss. This provided no real explanation of the observed facts, some of which are, indeed, directly opposed to an interpretation on the lines of "presence and absence." Since this subject is one of great importance for all students of genetics and evolution, it may be of some value briefly to review it in the light of a new theory recently advanced in this journal by Dr. R. A. Fisher (1928a, 1928b).

This worker points out that the response of the organism to recurrent mutations may be modified by selection of the wild-type gene complex. Assuming that all heterozygotes were originally intermediate, there would be a tendency for those to survive whose gene complex reacts in the most favorable manner with any new mutation. Thus if the effects of a gene were disadvantageous they would become increasingly recessive, while if beneficial they would shift towards complete dominance, owing to the selection of those heterozygotes in which they were most markedly developed.

The view here outlined is supported by experimental work on *Drosophila* and rodents, and by the behavior of factors which have arisen during the formation of the domestic breeds of poultry, and the "Sea Island" from the other New World cottons (Fisher, *loc. cit.*). It has, however, been challenged on mathematical grounds by Professor Sewall Wright (1929), whose calculations lead him to infer that selection of this kind is not sufficiently powerful to produce an appreciable effect except in artificial conditions. Dr. Fisher's own calculations (Fisher, 1929), published in answer to those of Professor Sewall Wright, lead to the opposite conclusion, and suggest that the result of such selection can not safely be neglected.

Some further evidence in support of Dr. Fisher's contention may, however, be obtained from a study of the manifold effects of single genes. In insects the influence of selection on such a character as body-color is probably considerable, since this might seem to have an obvious survival value in nature. The same may be true of eye-color in so far as it could affect the efficiency

of vision. But it has been shown (Dobzhansky, 1927, p. 383) that the allelomorphic genes for wild-type, sooty and ebony body-color and for the white-eye series in *Drosophila* have a secondary effect on the shape of the spermatheca. This is a small but exceedingly constant change in proportion; it is practically uninfluenced by external conditions, which, however, alter the absolute size of this organ very considerably (*loc. cit.*, pp. 339–342). Such a character can scarcely be controlled by direct selection. It is therefore a somewhat impressive fact that while these genes are, with the partial exception of ebony, almost completely recessive in regard to body-color and eye-color, yet in the shape of the spermatheca the heterozygotes are intermediate. Thus it would appear that selection has been able to produce the recessive condition in respect of certain disadvantageous characters without influencing the harmless secondary effects associated with them.

The only explanation of dominance previous to the publication of Dr. Fisher's theory was the long discussed physical one of partial or complete loss (or inactivation) of genic material at mutation, and Professor Sewall Wright falls back upon this in his criticism of it. Not only does the loss hypothesis lead to an evolutionary impasse when accepted as a general principle, but it can not be too strongly urged that as such it is untenable in face of the proved fact of reverse mutation. This demonstrates conclusively that germinal changes, other than losses, do in fact occur in genetic work, whether they be gains or alterations in gene structure.

On the other hand, it can hardly be doubted that many mutations are in the nature of losses partial or complete. Partial and progressive loss appears a probable explanation of the series of multiple allelomorphs at the white-eye locus in *Drosophila melanogaster*. This is strongly supported by the demonstration (Mohr, 1919, 1924, 1929) that the Notch-8 deficiency of 3.8 units, including within it the white-eye locus, produces the "ultra-white" effect, which represents the last term in this series. Notch-8 is a typical deficiency, of which many are known. It depends on an actual loss or inactivation of chromatin material, leading to exaggeration and pseudodominance (of the recessive genes in the corresponding range of the homologous chromosome), to an inhibition of crossing-over within the region concerned and to a homozygous lethal effect. These phe-

nomena are associated with the haploid-4 chromosome deficiency, which can be demonstrated cytologically (Bridges, 1921). The evidence in favor of genic loss as a basis of mutation is thus very convincing in the white-eye series, where progressive mutations at the same locus lead to a graduated diminution in pigment production, whose final term is a demonstrable deficiency.

But the impossibility of appealing to the loss itself as the basic factor in producing the recessive condition is here made apparent, for the effect of these same genes on the shape of the spermatheca is not recessive but, as previously mentioned, the heterozygote is intermediate. At present this fact can be accommodated only by the hypothesis of Fisher, which is in accord with what is known of the modifiable phenotypic effects of genes, and brings dominance into line with such phenomena as those presented by the epistatic and other interesting factors.

Further, it has long been known (Morgan and Bridges, 1913) that heterozygous white has a diluting effect not only in combination with the lighter allelomorphs of its own series, but also in flies homozygous for other light eye-color genes situated elsewhere in the chromosomes. This is even more marked in "ultra white," produced by the Notch-8 deficiency. But it has been demonstrated that here the dilution is not due to the sectional change as a whole, but only in so far as the white-eye locus is affected by it (Mohr, 1924, pp. 145–146). We therefore have a clear proof that complete loss of genic material at a single locus can, in certain gene combinations, lead to semidominance.

The mere assumption of partial or complete loss of genic material at mutation can not in itself provide an explanation of the recessive condition. It has perhaps been pointed out most clearly by Professor E. S. Goodrich (1924, pp. 61–62) that characters are the result of the interaction of genes with the environment. But the environment is both external and internal. Alter either the external environment or the gene complex and the effect of a given gene may be changed. Dominance is one of the phenotypic effects of a gene, and as such it is not the necessary prerogative of that gene itself but of its interaction with a modifiable environment. Why then should a loss, rather than a gain or a change, lead to the recessive state?

The important results obtained by Stern (1929), "Über die additive Wirkung multipler Allele," do, however, suggest a possibility which might lead some loss mutations to be recessive,

for he was able to reproduce the normal dominant condition by accumulating the recessive multiple allelomorphs of the character "bobbed" *bb* ("kurzborstig") carried in extra Y chromosomes. The amount of genic material present at a locus can therefore have a quantitative effect. Should this act up to a saturation value, beyond which the phenotype could not be altered, complete dominance would result, provided that this value be exceeded in the heterozygote. Even so the possible effect of selection must be admitted, since it is to be expected that the saturation level would respond to changes in the gene complex.

Since the above remarks were written a valuable and illuminating paper has been published by Dr. J. B. S. Haldane (1930), who points out the strong probability that the genes do in fact often act up to a saturation value. It is clear that mutations from a gene which has reached saturation level are detectable only when they take place in a minus direction, and that a number of phenotypically inseparable mutations of higher value might become available for recombination. Haldane suggests that those heterozygotes will be selected in which loss mutations are balanced by allelomorphs so much above the saturation level that the combined action of the pair is not reduced below it. There would thus be no loss of activity, and dominance would result. At the same time the possibility previously suggested must be borne in mind, that the saturation level itself may be modified by selection.

Haldane also raises the difficult problem of polymorphism, and in particular those instances where the mutant types are dominant to the normal form. I have discussed this question with my friend Dr. Fisher and owe the following suggestions to him.

The necessary condition for the development of polymorphism is that the frequency ratio of the members of some allelomorphic pair should be in stable equilibrium. That such equilibrium is maintained is shown by many examples in nature, where the different forms of a polymorphic species remain relatively common over a wide area. Thus the reef heron, *Demiegretta sacra* Gmel., is found from India to Australia. The adults of both sexes are either slaty gray or white, and the two types are found together indiscriminately. The young are pied, and every intermediate stage of plumage occurs among them, but the adults are

always either pure white or all slaty (Baker, 1929, pp. 351–352). According to Stresemann (1926, p. 381) the slaty form is dominant, but he gives no authority for this statement. It seems clear, however, that a simple relationship involving dominance exists between them.

It has been demonstrated (Fisher, 1922) that stable gene ratios between members of a Mendelian factor pair can occur only if selection favors the heterozygotes, so that there must always be a balance of selective advantage in these cases. Now there is some evidence that polymorphism is associated with partial sterility or inviability of the homozygous dominants. Thus the mimetic butterfly, *Papilio polytes*, L., has three female forms, of which one (the non-mimetic) is recessive, while the two mimics are dominants, two factor pairs being involved. Fryer (1913), in his breeding experiments with this species, encountered numerous sterile unions, and it has already been suggested (Fisher, 1927) that the most obvious explanation of this condition is a greater fertility of the heterozygotes compared with the homozygotes. Gerould (1923) in his work on the dominant white female in several *Colias* species, encountered great difficulty in obtaining the homozygous white type, owing to the occurrence of a closely linked lethal factor. This must be held in equilibrium, since apparently it is not rare in natural conditions.

In such instances as these the heterozygous types must be quite common in the population, instead of forming a minute fraction of it (perhaps one in 10^4 or so) as they do in general. This condition is thus very favorable for dominance modification, as the heterozygotes will be particularly quick to convert themselves to the most advantageous type.

SUMMARY

1. The hypothesis of Fisher postulates that heterozygotes are originally intermediate, and that dominance arises through selection of the gene complex, those types of heterozygotes being preserved which react most favorably with a given recurrent mutation.

2. This view is supported by work on *Drosophila*, rodents and other forms, cited by Fisher, and by the researches of Dobzhansky on the multiple effects of single genes.

3. Mutations are due (*a*) to changes in gene structure, which

may be alterations or additions of material, and (*b*) to actual losses of genic substance, whether partial or complete.

4. Partial or complete loss of genic material provides no explanation of the dominant and recessive conditions.

5. Demonstrable genic losses do not, therefore, always lead to the recessive state.

6. Only in so far as loss mutations are quantitative in effect and are associated with a saturation value which is exceeded by the heterozygote can they lead to dominance.

7. All the mutations from a gene which exceed such a value will be indistinguishable. But it has been suggested by Haldane that those will be selected which, in combination with a given mutation in the minus direction, maintain the combined value of the pair above saturation level.

8. It is, however, to be expected that the level of any saturation value could be altered by selection operating on the gene complex.

9. Polymorphism may be associated with dominant mutant forms. It must always depend upon a stable equilibrium between the frequency ratios of some allelomorphic pair. Such stable equilibrium can be maintained only if selection favors the heterozygotes. These will consequently form a significant proportion of the population, leading to a condition singularly favorable to the production of dominance in the manner suggested by Fisher.

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MEIOTIC BEHAVIOR OF TRIPLOID OENOTHERAS

THE triploid forms of Oenothera which are referred to by Oenothera geneticists as *semigigas* or "heroes" seem to present a rather intricate situation in some of their cytological phenomena. Very recently their synapsis and meiosis have been the subject of discussion between Darlington (1930) and Gates (1930), the controversy arising from the novel observation reported by Catcheside (1930) that a circle of 21 chromosomes was formed in a triploid plant. For the past two years I have been conducting studies on the genetics and cytology of triploid Oenotheras, and as their meiosis becomes an issue of lively interest I here report observations which have a bearing on the point under discussion and which constitute preliminary announcement of my findings in this phase of my investigations. My early observations were made in the fall of 1928 on 15 triploids which Dr. Shull turned over to me and the origin of which he has described in a recent paper (1929). I have since then made cytological analyses of the pollen mother cells of triploids in the summers of 1929 and 1930. The triploids studied in the last two years were mostly derived from pure *Lamarckiana.* My observations have been based on paraffin sections as well as on smear preparations. Materials for paraffin were fixed in Allen's modification of Bouin's fluid and stained with Heidenhain's iron-hematoxylin. The smear preparations

of pollen mother cells were fixed in chromo-acetic-formalin and stained with iron-brazilin according to the procedure found suitable to Oenothera buds by the writer (1930 *a*). My microscopic slide preparations have been examined both by Professor E. G. Conklin, under whose supervision I have acquired my cytological technique, and by Professor G. H. Shull, under whose direction I have been conducting this research.

In triploid microsporogenesis it can be frequently observed that rows of pollen mother cells in different phases of mitosis occur in the same bud. Not infrequently also various stages of cell division are found even in a single anther segment or loculus. This condition enables one to trace with accuracy the various phases of meiosis. Microscopic slide preparations, particularly the paraffin sections, are therefore available which show the stages from early presynizesis up to the reconstitution of daughter nuclei. I have observed that the leptotene threads issuing from the synizetic knot are single in nature as evidenced by the presence on them of a single row of chromomeres. Then in the second contraction stage the pachytene threads also remain single, an indication that there would result a univalent spireme. Later through contraction and condensation of these chromatic threads the chromosomes emerge as single segmented bodies invariably connected end to end, and undoubtedly conjugation has occurred in telosynaptic fashion. The arrangement of chromosomes and their configurations at diakinesis is in many instances, especially the Y-shaped (trivalent) chromosomes, in agreement with "Trivalentbildung" of Håkansson (1926), and it confirms the triple associations and pairing of chromosomes which were made out by Darlington (1930) on Catcheside's slides.

In my triploids are shown two or more chains open or closed to form rings, Y-shaped trivalents, single pairs of chromosomes forming bivalent rings and unpaired chromosomes. The ring pairs or bivalent rings generally encircle the long axis of the chain. The most significant of these configurations is the trivalent groups which are formed in various shapes, the predominating one being the Y-shaped. All prophase spiremes seem to be characterized by the presence of trivalents. The open chains are often branched at one end with from 1 to 2 chromosomes in a branch. In no case could it be observed or even inferred that

the 21 chromosomes were all united in a single circle.[1] Paraffin sections and uncut cells of smear preparations gave essentially identical results. The smear preparations showed especially clear figures of the presynizetic and postsynizetic stages. The various types of chromosome configurations persist in the formation of multipolar or bipolar spindles at meiotic metaphase. It is quite difficult to make out what takes place between the dissolution of nuclear membrane and the formation of spindle fibers in heterotypic metaphase. At metaphase plate the chromosomes align themselves in the characteristic zigzag manner of other Oenotheras, and the plate shows two or more circles or chains interlacing with one another. In anaphase the chromosomes seem to orient themselves at haphazard with respect to the poles to which they move. In this chromosome separation two members composing the trivalent move to one pole and the third to the other. The strands or the linin connections between the chromosomes may be clearly traced among spindle fibers during this separation. As is usual in polyploid meiosis there are lagging chromosomes in these triploids. Cases were seen where as many as seven chromosomes were lagging in the equatorial plate while 14 chromosomes were moving to the poles. The most frequent segregation of chromosomes in the triploids under report is 10 going to one pole and 11 to the other. Next in frequency is where 9 move to one pole and 12 to the other. In very few isolated and seemingly vague cases was seen a disjunction of 8 and 13. Chromosomes which lagged often disintegrated, resulting in the formation of an unequal number of chromosomes in daughter cells in homotypic mitosis. It seems apparent that the high percentage of pollen sterility in Oenothera triploids is mainly due to the irregular and unequal distribution of chromosomes in the final division of sporogenesis. Genetical evidence which indicates that the offspring of triploids have a wide range in chromosome number is familiar to students of the subject. My studies in this respect, which need not be detailed here, show the offspring of triploids with a range of 14 to 21 chromosomes.

The occurrence of trivalents, first reported by Håkansson (1926) and indisputably confirmed in the present studies, of

[1] Since this was written and after my letter reporting some of the same observations was published in *Nature* (1930 *b*), Professor Gates has written me that Mr. Catcheside has made a further study of triploidy and has come to conclusions which are similar to mine.

rings of chromosomes attached end to end, of bivalent and univalent chromosomes suggests at once the inadequacy of some of the hypotheses so far advanced to account for chromosome linkage. According to one hypothesis chromosome rings indicate heterozygosity. On this assumption it is difficult to imagine how such configurations as trivalents and ring pairs may be found associated with two or more chromosome chains. Another hypothesis assumes that "the phenomenon of segmental interchange is probably at the basis of circle formation in Oenothera" (Cleland and Blakeslee, 1930). A third hypothesis long held by Professor Shull as a favorite one of the several possibilities and recently suggested by Illick (1929) assumes chromosomal configurations to be the result of the presence of certain genes or combinations of genes just as other physical characters are known to be produced. This hypothesis considers chromosome linkage an inherited genetic character and not dependent on either harmony between homologous chromosomes or the lack of harmony in non-homologues. The recent work of Weier (1930) comes to the unique conclusion that the development and association of 12 chromosomes in a chain in *Oe. Lamarckiana* might just as well be assumed an association of non-homologues as of homologues, implying that they undergo no synapsis, a condition which he terms "asynapsis." While this assumption is in agreement with some breeding results of Oenothera, nevertheless it tends to ignore the generally observed behavior of chromosomes during meiosis.

The triploids here reported have probably arisen through the fertilization of an unreduced egg by a reduced sperm as pointed out by Shull (1929). To interpret the diverse configurations here observed will involve a description of a long series of cytological events which the writer will undertake in the fuller report of these investigations which he hopes soon to make. The work is being extended by cytological analyses of about a dozen groups of triploids which have appeared among the segregates of diploid crosses and selfed lines of several different species of Oenotheras in Dr. Shull's experimental cultures. These later triploids appeared in various species and combinations involving *Oe. franciscana, Oe. ammophila, Oe. biennis, Oe.* mut. *bullata, Oe.* mut. *fasciata* (from *erythrina*), and double-flowered *Oe. Lamarckiana.* None of the triploids studied have been derived from a cross in which *gigas* or another triploid was

one of the parental forms, but all have arisen *de novo* by mutational process.

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ON THE INFLUENCE OF TEMPERATURE ON THE PROCESS OF MUTATION, WITH REFERENCE TO GOLDSCHMIDT'S DATA

THE periodical *Biologisches Zentralblatt* published in 1929 a paper by Professor R. Goldschmidt entitled "Experimentelle Mutation und das Problem der sogenannten Parallelinduktion. Versuche an Drosophila."

In this work the author states that he succeeded in obtaining a very high percentage of mutations by applying a temperature of 37° C. to larvae 3 to 6 days old. Together with this high percentage of mutations, two more points of note attract attention.

(1) The similarity in phenotypical variation of flies subjected to high temperature and of their progeny (thus, in P there arose flies that were phenotypically e^s, and later in crosses of their progeny arose the same e^s). On this ground the author speaks of "Zufallsparallelinduktion."

(2) The specificity of the mutations obtained. All the mutations named in Goldschmidt's work are located by him in the third linkage group, except the cases in which "white" and "rolled" appeared.

This leads the author to speak of temperature as a specific agent, causing the process of mutation specifically in the third chromosome.

Our laboratory is studying the influence of external agents on the rate of mutation in connection with other questions on the nature and structure of the gene. It was therefore with an especial interest that it encountered the work of Goldschmidt. This work seemed to be a sign-post on the way leading to a knowledge of the method for calling forth or regulating the process of mutation at a given point in the chromosomal apparatus. Before Goldschmidt's work no external agents specifically influencing given points in the chromosomes were known to genetics. All external agencies influencing the mutation process and also the crossing over process acted on the entire chromosomal apparatus as a whole.

After the publication of Goldschmidt's work, it seemed as if geneticists had received a very perfect instrument for directing the process of mutation. We deemed it therefore necessary to repeat the work done by Goldschmidt, keeping as much as possible to his method in all its particulars.

We undertook two series of experiments. The methods used for both series were identical. The material used was the line "radius incompletus" (a gene of the third chromosome found in studying the Caucasian population of *D. melanogaster*). Larvae from 5 to 6 days old were placed in a thermostat at a temperature of 37° C. for 12 hours, after which they were again placed at a temperature of 25° C. The examination of flies developed from the larvae which had been subjected to the action of high temperature showed many divers modifications. All data relative to the modification of the parental generation (P) have been included in Table I.

It can be seen from this table that the temperature exerted an influence on all the characters of the fly: eyes, bristles, wings, etc. It is further to be remarked that, in addition to the variations which are tabulated, a general darkening of color was observed in all the flies developed from the larvae that were subjected to high temperature. Like Goldschmidt, we also

TABLE I
MODIFIED P FLIES

Experiment	Number of bottles in P	Number of P flies		Single singed bristles		Kidney-shaped eyes		Absence of single bristles		Bristles of minute type		Abnormal distribution of microchaetes		Rough eyes		Incised wings		Very dark body-color		Reduction of third pair of legs		Total of modified flies		Per cent. of modified flies
		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	
1	18	236	260	9	7	3	4	5	0	0	0	0	0	2	0	2	0	0	2	0	2	21	15	7.3
2	20	360	328	17	18	8	6	2	4	3	0	2	0	1	1	1	0	1	0	0	0	35	29	9.3

found a certain death-rate resulting from exposure to high temperature; this can be seen from the fact that a culture gave on the average about 30 flies, against 40 flies in the control series.

The pair-matings made on purpose to obtain the F_1 showed a high percentage of sterility among the flies: of 188 matings only 98 gave offspring. A control series showed no departure whatever from type.

The results of the first and the second series of experiments being the same, they will be referred to together. The examination of the F_1 as shown in Table II did not result in the detection of any variations.

TABLE II
F_1 FLIES

	Number of bottles	Number of r.i. flies		Number of modif. flies	
		♀	♂	♀	♂
Experiment	98	2661	2531	0	0
Control	32	702	651	0	0

Not only did the normal flies give only normal progeny (except in the cases cited below) but the phenotypically modified ones showed no variations in their offspring.

We shall not present here all the results of the experiments made in crossing the modified flies of the P generation, but shall only enumerate a few of them, the rest not having brought forth anything new.

It must be noted that the process of the work showed the line "radius incompletus" to be heterozygous for the gene

"white ocelli," and that the examination of F_1 both in the experiments and in the control series showed w-o flies. Further, flies with roof wings and with dachs legs were found, both in the experiments and in the control. Both these last variations were found upon examination to be not inheritable. The F_2 as can be seen from Table III also showed no morphological variations.

TABLE III

Type of mating	Number of bottles	F_1	F_2
♀ kidney-shaped eyes × ♂ kidney-shaped eyes	4	Normal	Normal
♀ kidney-shaped eyes × ♂ +	4	"	"
♀ + × ♂ kidney-shaped eyes	4	"	"
♀ singed bristles × ♂ singed bristles	1	"	"
♀ very dark body-color × ♂ +	1	"	"
♀ + × ♂ very dark body-color	1	"	"
♀ rough eyes × ♂ rough eyes	1	"	"

TABLE IV

F_2 FLIES

	Number of bottles	Number of r.i. flies		Number of modif. flies	
		♀	♂	♀	♂
Experiment	129	2788	2607	0	0
Control	30	629	608	0	0

Among the F_2 flies examined, 3 cases showed deviation from type in regard to the sex-ratio. Two of them were found in the experiments, one in the control. All the three cases showed a lethal sex-ratio and are at present under examination, but it is evident that whatever the answer should prove to be, it can have no influence on the results of the investigation.

Thus it is evident that the present work has not confirmed the data of the work of Goldschmidt. We have not the least doubt that the influence of temperature is capable of accelerating the process of mutation. Muller has shown with almost absolute certainty that the influence of high temperature raises the frequency of lethal mutations in *Drosophila melanogaster*. We are certain that it is also possible to show by duly organized experiments the influence of high temperature on the origination of visible mutations.

But at present it is impossible to speak of the influence of high temperature applied to larvae 5 to 6 days old as raising the mutation rate—at least, with the data obtained by us as the only basis.

We once more repeat that the present paper must be regarded only as an attempt to test the results described by Goldschmidt. We are at present obtaining an F_3; but as Goldschmidt in his paper says nothing about the F_3, the data gained in this way will not be fit for comparison with his.

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